The Journal of
CLINICAL ENDOCRINOLOGY
AND METABOLISM

Table of Contents for September 1955

Sweat, Max L., with the technical assistance of Lipscomb, Marion D. Adrenocorticosteroids in Peripheral and Adrenal Venous Blood of Man 1043

Hetzel, Basil S.; Schottstaedt, William W.; Grace, William J., and Wolff, Harold G., with the technical assistance of Plescia, Alba; Whittington, John M., and Geoghan, Barbara. Changes in Urinary 17-Hydroxycorticosteroid Excretion During Stressful Life Experiences in Man 1057

Franksson, Curt, and Gemzell, Carl A. Adrenocortical Activity in the Preoperative Period 1069

Wallace, Eleanor Z.; Christy, Nicholas P., and Jailer, Joseph W. Clinical Application of the Simplified Silber-Porter Method for Determining Plasma 17-Hydroxycorticosteroids 1073

Kenny, A. J. Extractable Glucagon of the Human Pancreas 1089

Payne, H. W., and Latour, J. P. A. Quantitative Estimations of Endometrial Glucagon, Using the Anthrone Method 1106

Danowski, T. S.; Schwartz, R.; Rhodes, G. J.; Yoder, R. D.; Puntereri, A. J., and Mateer, F. M. Serum Protein-Bound Iodine Levels Following Administration of Thyroxine in Various Diseases 1116

Wiener, Roslyn; Iannaccone, Angelo; Eisenberg, Julius; Griboff, Solomon I.; Ludwig, Arthur W., and Soffer, Louis J. Influence of Hormone Therapy on Body Fluids, Electrolyte Balance and Mucopolysaccharides in Myxedema 1131

Tsumori, Himeo; Jensen, Esther; Hunnicutt, Arthur J.; Foreman, Nadine, and Kinsell, Laurance W. Juvenile Hyperparathyroidism in Association With Peptic Ulcer 1141

Teaching Clinic

Lisser, H. An Adrenal Tumor, Clinically Doubtful; Diagnosis Established by Extraperitoneal Pneumography 1144

Letters to the Editor

Maengwyn-Davies, Gertrude D., and Weiner, Ruth. Excretion of 17-Hydroxycorticosteroids During the Menstrual Cycle 1150

Lampe-Hintzen, D. A. V. M., and Huis in 't Veld, L. G. Atypical Porter-Silber Reaction After the Administration of Sulfamerazine 1152

The Endocrine Society

The 1955 Postgraduate Assembly in Endocrinology and Metabolism 1155

The 1956 Awards 1156

Book Register 1158

For GLUCURONIDASE studies **specify SIGMA**

PHENOLPHTHALEIN GLUCURONIDE

Available as: Soluble Free Acid; Prepared Standard Solution; Cinchonidine Derivative

REDUCED PRICES NOW IN EFFECT:
CINCHONIDINE DERIVATIVE NOW ONLY $35 per gram;
FREE ACID, NOW $70 per gram.

The Sigma "Cooperative Allowance" is readily available where budgets are inadequate. Call or write for details, if you need help. Mr. Dan Broida, EVergreen 3-3529.

SIGMA
CHEMICAL COMPANY

4648 EASTON AVENUE, ST. LOUIS 13, MO., U.S.A.
MANUFACTURERS OF THE FINEST BIOCHEMICALS AVAILABLE

An Authorized Binding for JOURNAL OF CLINICAL ENDOCRINOLOGY AND METABOLISM

Arrangements have been made for subscribers to have their journals bound into distinctively designed books.

Twelve issues, January through December, bound in best grade green washable buckram, imprinted with your name on cover, cost but $3.45 per volume.

Bound journals serve as an immediate reference for research and information. Properly displayed, they create a psychological impact on the patient, implying the time and effort spent to keep up-to-date on the most modern techniques and treatments.

Ship journals parcel post. Within thirty days after receipt, bound volumes will be shipped prepaid anywhere in the U.S.A. Full remittance must accompany order.

Publishers' Authorized Bindery Service
(Binders of all Journals)
5811 West Division St., Chicago 51, Illinois

FUNDAMENTALS OF BIOCHEMISTRY IN CLINICAL MEDICINE

By
NIELS C. KLENDSHOJ, M.D.
Assistant Professor of Pathology
University of Buffalo

"Obviously designed to help the busy doctor review biochemical principles which have grown hazy with time, as well as to furnish a synthesis of important recent advances in the field."—*GP*

279 pages *18 illustrations*

Sent on approval, $7.75, postpaid

CHARLES C THOMAS · PUBLISHER
Springfield · Illinois

The Journal of
CLINICAL ENDOCRINOLOGY
AND METABOLISM

VOLUME 15 SEPTEMBER, 1955 NUMBER 9
Copyright 1955 by the Endocrine Society

ADRENOCORTICOSTEROIDS IN PERIPHERAL AND ADRENAL VENOUS BLOOD OF MAN*†

MAX L. SWEAT, PH.D.
with the technical assistance of MARION D. LIPSCOMB
The Department of Physiology, Western Reserve University School of Medicine, Cleveland, Ohio

ADVANCES in steroid methodology make possible a direct approach to the problem of the nature of the secretion of the human adrenal cortex. Two experimental studies in which adrenal venous blood was analyzed (1, 2) indicate that 17-hydroxycorticosterone and corticosterone are components of the steroid mixture elaborated by the human adrenal. These two steroids have also been reported to be present in the peripheral blood of man (1–11).

The present communication is an extension of our preliminary report (1) on the subject.

METHODS

Blood samples were extracted with chloroform, partitioned between 70 per cent ethanol and petroleum ether and chromatographed on silica gel (12). Effluent fractions from chromatography were quantitatively analyzed for corticosterone and 17-hydroxycorticosterone by the phenylhydrazine (13) and fluorescence techniques (14).

Chemical identification studies were conducted on the combined ef-

Received for publication December 31, 1954.

* This investigation was supported by a research grant (A-331) from the National Institute of Arthritis and Metabolic Diseases of the National Institutes of Health, U. S. Public Health Service.

† A preliminary report (1) was read at the Thirty-Seventh Annual Meeting of the American Physiological Society, April 6 to 10, 1953.

fluents obtained from the corticosterone and 17-hydroxycorticosterone regions.

Subjects treated with ACTH received either a single dose of 15 units intravenously or 40 units by infusion over four hours. Peripheral blood was obtained from an antecubital vein. Adrenal venous blood was obtained by cannulation of an adrenal vein immediately prior to adrenalectomy.

RESULTS AND DISCUSSION

Figure 1 depicts the pattern obtained after chromatography and fluorescence analysis of a mixture of 1 μg. of corticosterone and 1 μg. of 17-hydroxycorticosterone. Fractions 1 to 3 are the effluents from three 5-ml. quantities of chloroform; fraction 4 is the effluent from one 5-ml. quantity of 0.5 per cent ethanol in chloroform; fractions 5 to 8 are the effluents from four

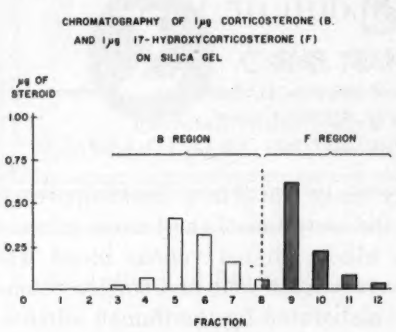

FIGURE 1

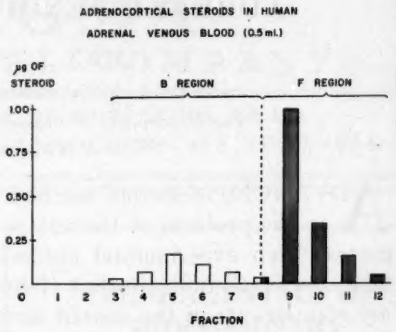

FIGURE 2

5-ml. quantities of 1.0 per cent ethanol in chloroform; and fractions 9 to 12 are the effluents from four 5-ml. quantities of 4.0 per cent ethanol in chloroform. It will be seen in the figure that corticosterone reached a peak in fraction 5 and faded to a minimum detectable quantity in fraction 8. 17-Hydroxycorticosterone reached a peak in fraction 9 and "tailed out" into fraction 12.

A pattern similar to that of a mixture of corticosterone and 17-hydroxycorticosterone was obtained from the chromatography of human adrenal venous blood (Fig. 2.).

Human peripheral blood from untreated subjects and from subjects treated with ACTH had essentially the same chromatographic patterns as those of adrenal venous blood and of standard mixtures of corticosterone and 17-hydroxycorticosterone (Fig. 3). The least polar of the fluorescent substances in adrenal venous blood and in peripheral blood of untreated and ACTH-treated subjects reached a peak in fraction 5, as did cortico-

sterone. The most polar of the fluorescent substances in blood from the three sources showed a peak in the same fraction as 17-hydroxycorticosterone, namely, fraction 9.

Figure 4 depicts the relative quantities of substances reaching a peak in fractions 5 and 9 in peripheral blood from normal subjects, from normal subjects treated with ACTH and from a patient with Cushing's syndrome. Also in the figure are data on adrenal venous blood from a patient with carcinoma of the breast and from a patient with Cushing's syndrome.

Table 1 lists the concentrations of fluorescent corticosteroids in the blood of normal subjects and in the blood of patients with various endo-

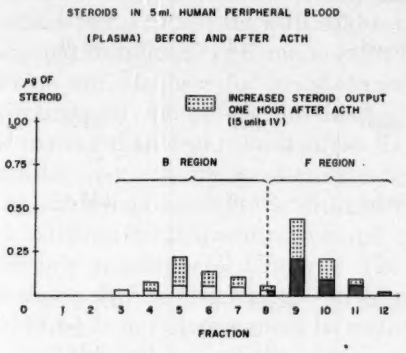

FIGURE 3

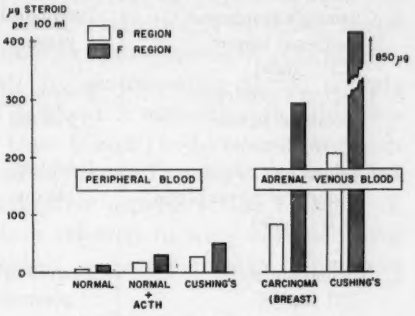

FIGURE 4

crinopathies. The analyses of three samples of adrenal venous blood are listed in the table. As pathologic examination of the adrenal glands from the 2 patients with breast carcinoma revealed no gross or microscopic abnormalities, it is possible that the quantities of corticosterone and 17-hydroxycorticosterone secreted from these glands are representative of the quantities of these steroids secreted from the adrenal glands of normal subjects under operative stress. However, the possibility of metabolic aberrations not associated with morphologic changes in the adrenals of those patients cannot be ruled out. Adrenal venous blood collected from the patient with Cushing's disease associated with bilateral hyperplasia contained the highest concentrations of the two steroids we have observed in human adrenal venous blood. The highest concentrations of 17-hydroxycorticosterone and corticosterone-like steroids observed in peripheral blood were likewise obtained from a patient with bilateral adrenal hyperplasia (Subject 6 of Table 1). The peripheral steroid levels listed for the 2 patients with adrenal tumors are significantly lower than the values listed for the patient with adrenal hyperplasia. The concentrations of 17-hydroxycorticosterone in these latter patients were significantly

TABLE 1. FLUORESCENT STEROIDS IN HUMAN BLOOD

Subject	Sample	Fluorescent steroids (μg./100 ml.)	
		B region	F region
1. Breast carcinoma, (G., female)	Adrenal venous whole blood	32	319
2. Breast carcinoma, (E.B., female)	Adrenal venous whole blood	170	330
3. Cushing's syndrome, (bilateral hyperplasia, S.M., female)	Adrenal venous whole blood	203	850
4. Cushing's syndrome, (adrenal tumor, J.P., male)	Peripheral blood plasma	7.2	33.0
5. Cushing's syndrome, (adrenal tumor, E.W., female)	Peripheral blood plasma	3.5	21.0
6. Cushing's syndrome, (bilateral hyperplasia, J.B., male)	Peripheral blood plasma	18.3	135.0
7. Normal subjects (21 males)	Peripheral blood plasma	4.3±2.3* ±0.5†	10.8±2.6* ±0.6†
8. Normal subjects+ACTH	Peripheral blood plasma	Twofold to fivefold increases	Twofold to fivefold increases
9. Addison's disease:			
W.J. pre-ACTH	Peripheral blood plasma	4.3	7.2
post-ACTH‡		7.6	9.8
J.V. pre-ACTH	Peripheral blood plasma	5.1	9.8
post-ACTH‡		6.2	6.6

* Standard deviation.

† Standard error of the mean.

‡ Dose=40 units of ACTH over a period of four hours. Samples obtained immediately after end of infusion.

higher than the normal range; the concentrations of corticosterone-like steroids were within the normal range.

It will be noted in the table that the quantities of 17-hydroxycorticosterone and corticosterone-like steroids in the peripheral blood of 2 pretherapy patients with Addison's disease in crisis were within the same range as is found in normal unstressed subjects. However, when ACTH

was administered to these patients, no significant increase in the concentration of either 17-hydroxycorticosterone or corticosterone-like steroids was observed. This indicates a distinct difference between these patients and normal subjects. If adrenal tissue was present in these patients, apparently it was secreting at maximum capacity. Nelson and Samuels (15) have reported that 30-ml. samples of peripheral whole blood from 3 patients with Addison's disease contained no 17,21-hydroxy-20-ketosteroids.

In interpreting the observation that the blood of patients with Addison's disease may contain quantities of corticosteroids comparable to those observed in normal unstressed subjects, the following possibilities should be considered: 1) Patients with Addison's disease may be in various states of adrenal insufficiency. Some may have small quantities of secreting tissue which are adequate for maintenance under circumstances of optimal environmental conditions, but inadequate for maintenance during periods of increased environmental stress. 2) The blood of patients with Addison's disease may contain substances other than F and B which interfere with the analytical method. Additional studies on methodology are required to establish both quantitative and qualitative aspects of the presence of steroids in peripheral blood. 3) The data referred to were obtained in 2 patients. Before conclusions can be reached, a greater number of patients with Addison's disease must be studied.

In Table 2 the quantities of corticosterone and 17-hydroxycorticosterone in the adrenal venous blood of a patient with carcinoma of the breast are compared with the quantities of these two steroids in samples of peripheral blood plasma collected before operation, and in peripheral blood collected simultaneously with adrenal venous blood during operation. It will be noted that the same ratio of corticosterone to 17-hydroxycorticosterone observed in the adrenal venous blood sample was also observed in all peripheral blood samples collected from this patient. Whether the relation

TABLE 2. CORTICOSTEROIDS IN PERIPHERAL AND ADRENAL VENOUS BLOOD FROM PATIENT WITH CARCINOMA OF THE BREAST (μG./100 ML.)

Sample	B region	F region	Ratio (B:F)
Peripheral blood plasma			
pre-ACTH	8.3	16.8	1:2.0
post-ACTH	23.7	45.0	1:2.0
drawn simultaneously with adrenal venous blood	15.4	31.7	1:2.1
Adrenal venous blood	170	330	1:1.9

of corticosterone to 17-hydroxycorticosterone is the same for adrenal venous blood and peripheral blood plasma in all individuals will require further investigation. It will also be noted in the table that the peripheral blood plasma collected during the operation contained less corticosteroid than the sample collected after administration of ACTH. As it is not yet clear whether the utilization of steroids is increased during stress, this observation cannot be interpreted to mean that the stress of operation evoked smaller quantities of corticosteroids than the ACTH.

Figure 5 depicts the increase of 17-hydroxycorticosterone and the corticosterone-like steroid after the intravenous administration of 15 units of ACTH to 2 normal subjects.

Table 3 is a summary of the evidences obtained from this laboratory which support the concept that the fluorescent steroids in human adrenal

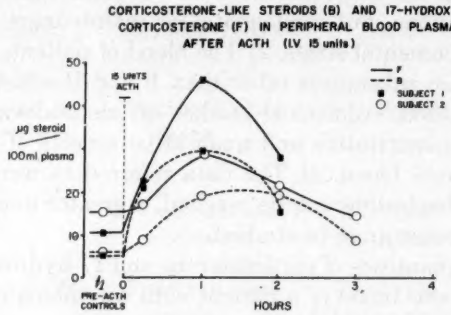

FIGURE 5

venous blood and human peripheral blood are corticosterone and 17-hydroxycorticosterone.

The emission of yellow-green fluorescence which corticosterone and 17-hydroxycorticosterone exhibit when treated with sulfuric acid, and the elution patterns obtained when these steroids are chromatographed on micro silica-gel columns have been described in detail elsewhere (12, 14). These tests, when considered separately, are of relatively high specificity; when they are combined in the analytical procedure, the degree of specificity is enhanced.

The fluorescence test appears to be relatively specific for corticosterone, 17-hydroxycorticosterone and 4-pregnene-11β,17α,20β,21-tetrol-3-one (2). Other tested corticosteroids exhibit much less intensity of fluorescence. Fluorescent steroids in the estrogen series are easily distinguished by chromatography.

The specificity obtained from the micro-silica-gel column is due to the relatively high polar properties of corticosterone and 17-hydroxycortico-

TABLE 3. EVIDENCE FOR THE PRESENCE OF HYDROCORTISONE AND CORTICOSTERONE IN HUMAN BLOOD

Test	Reference steroids		Blood steroids			
			Adrenal venous		Peripheral	
	B	F	B	F	B	F
Silica-gel chromatography	Peaks in fraction #5	Peaks in fraction #9	Peaks in fraction #5	Peaks in fraction #9	Peaks in fraction #5	Peaks in fraction #9
Paper chromatography			RF values same as for B	RF values same as for F		RF values same as for F
Phenylhydrazine absorption maximum	340 mμ	400 mμ	340 mμ	400 mμ		400 mμ
Ultraviolet absorption maximum	239	239	239	239		
Fluorescence	Yellow green	Yellow green	Yellow green	Yellow green	Yellow green	Yellow green
Ammoniacal silver nitrate	+	+	+	+		
Response to ACTH a. normal b. Addison's disease					a. increase b. little or no increase	a. increase b. little or no increase
Cushing's syndrome			increase	increase	increase	increase
Other evidences					Human adrenal secretes B and F	

sterone. The relative polarity of these and other steroids is presented in Table 4. Corticosterone is eluted in 1 per cent ethanol solutions of choloroform; F is eluted in 4 per cent ethanol. Of the steroids studied, those containing four or less oxygen atoms (androgens, estrogens, progestins and some corticosteroids) are eluted in fractions preceding the corticosterone fractions. Cortisone, dihydro and tetrahydro cortisones, and dihydro and tetrahydro 17-hydroxycorticosterones are eluted in the late 1 per cent ethanol and early 4 per cent ethanol fractions. 4-Pregnene-11β,17α,20β,21-tetrol-3-one is eluted in the 10 per cent ethanol fractions.

Of the steroids which have been identified in adrenal tissue and blood, those with polar properties most similar to corticosterone and 17-hydroxycorticosterone appear to be cortisone and the tetrahydro derivatives of cortisone and 17-hydroxycorticosterone. However, these compounds exhibit only a low degree of fluorescence and their presence does not present serious analytical problems in the analysis of either corticosterone or 17-hydroxycorticosterone. The possibility that large quantities of these low-fluorescence substances may account for the degree of fluorescence ob-

TABLE 4. KNOWN CORTICOSTEROIDS POSSIBLY PRESENT IN HUMAN BLOOD

Polarity on silica gel	Fraction No.	Per cent ETOH in $CHCl_3$	*= Highly fluorescent R = Reichstein's designation K = Kendall's designation + = Not tested on column (position estimated)
Extra high	14	10%	*4-Pregnane-11β,17α,20β,21-tetrol-3-one (E_R)
	13		Pregnane-3β,11β,17α,20β,21-pentol (A_R)+
High	12	4%	
	11		
	10		
	9		*17-Hydroxycorticosterone (F_K)
Moderately high	8	1%	Aldosterone+
			Cortisone
	7		Pregnane 3 (α or β), 11β,17α,21-tetrol-20-one (tetrahydro F compounds)
	6		Pregnane (3α or 3β), 17α,21-triol-11,20-dione (tetrahydro E_K compounds)
	5		*Corticosterone (B_K)
Intermediate	4	0.5%	17-Hydroxy-11-desoxycorticosterone (S_R)
			4-Pregnene-21-ol,3,11,20-trione (A_K)
	3		Allopregnane-3α,11β,21-triol-20-one+
Low	2	0%	Corticosteroids containing three or less oxygen atoms (desoxycorticosterone)
	1		
	0_2		Estrogens
	0_1		Androgens

served in peripheral blood is ruled out, as such quantities are easily detected by the phenylhydrazine reaction. Further, these substances tend to reach a peak in effluent fractions following the fraction in which there is a peak for corticosterone. Their presence in appreciable quantities would tend to induce "humps" in the tailing pattern of corticosterone.

The evidences presented from this laboratory (1) give strong support to the thesis that corticosterone and 17-hydroxycorticosterone are major steroid components of adrenal venous blood. The crystallization and infrared analyses of these two compounds from adrenal venous blood by Romanoff *et al.* (2) leave no doubt as to the identity of these substances.

Although rigorous tests such as crystallization and infrared analysis have not been applied to the 17-hydroxycorticosterone-like substance in

TABLE 5. PRESENT STATUS OF KNOWLEDGE OF CORTICOSTEROIDS IN HUMAN ADRENAL VENOUS AND PERIPHERAL BLOOD

I. *Adrenal Venous Blood*	F_K*	B_K
Identification	Sweat *et al.* (1)	Sweat *et al.* (1)
	Silica-gel column	Silica-gel column
	Fluorescence	Fluorescence
	Phenylhydrazine derivatives	Phenylhydrazine derivatives
	Paper chromatography	Paper chromatography
	Romanoff *et al.* (2)	Romanoff *et al.* (2)
	Crystals	Crystals
	Melting point	Melting point
	Infrared spectra	Infrared spectra

II. *Peripheral Blood* (μg./100 ml. plasma)

Investigators and methods used	17-Hydroxy-corticosteroids	A_K	B_K	B-like	E_K	F_K
Sweat *et al.* (1)				4.3±2.3† [21]**		10.8±2.6† [21]
Silica-gel chromatography						
Solvent partitioning						
Fluorescence						
Nelson *et al.*††						
Phenylhydrazine (16)	♂13±6† [91]					
Florisil	♀15±6† [29]					
Morris & Williams (4)		2–5 [7]	4–10.5 [7]		2.5–5 [7]	6.5–10.5 [7]
Polarography						
Partition chromatography						
Bush & Sandberg (5)				none or 2		10–12
Paper chromatography						
Silica gel						
Kassenaar *et al.* (7)	♂6.3±1.2†					
Phenylhydrazine	♀6.1±1.3†					
Solvent partitioning						
Porter & Silber (9)	13.3±6.2† [21]					
Phenylhydrazine						
Solvent partitioning						
Bondy & Altrock (10)	7.8±6.2†					
Phenylhydrazine						
Chromatography						
Weichselbaum & Margraf (11)	9.5±1.5† [42]			8.9±2.0 [29]		
Phenylhydrazine						
Mader-Buck reaction (17)						
Chromatography						
Solvent partitioning						

* K = Kendall's designation.
** Figures in brackets equal number of samples.
† Standard Deviation.
†† Nelson and Samuels originally reported values in terms of whole blood (4–10 μg./100 ml.).

peripheral blood, data accumulated from several groups of investigators (1–11) appear to leave little doubt that the substance is 17-hydroxycorticosterone. On the other hand, evidence for the presence of corticosterone in peripheral blood does not appear to be conclusive. The question remains whether the fluorescent material chromatographing in the corticosterone region is corticosterone, a corticosterone-like compound or a mixture of corticosterone and another fluorescent steroid. Further studies are in progress for the more definite identification of this substance in peripheral blood which shows a peak in fraction 5 of the micro-column.

The present status of the knowledge of corticosteroids in human adrenal venous and peripheral blood is depicted in Table 5.

The micro silica-gel fluorescence method possesses advantages in its relatively high sensitivity. In blood the lower limit of sensitivity for 17-hydroxycorticosterone is 0.5 μg. for the phenylhydrazine method (3), and 0.05 μg. for the fluorescence method. In the fluorescent procedure, background interfering substances are in large measure eliminated; thus distinct fluorescent peaks with relatively large increments above background levels are observed with fluorescent steroids. "Quenching effects" which are commonly observed in the fluorometric analysis of estrogens in urine extracts have not been observed in the silica gel-fluorometric technique for corticosteroids in blood.

Bliss *et al.* (16) compared the fluorescence technique, which specifically measures 17-hydroxycorticosterone, with the method of Nelson and Samuels (3), which measures the 17,21-dihydroxy-20-ketone side chain. The values were relatively close as determined by the two methods and these authors concluded that the only major compound in peripheral blood having a 17,21-dihydroxy-20-ketone side chain is 17-hydroxycorticosterone. However, Bayliss and Steinbeck (8) are inclined to the view that only one-third to one-half the total quantity of 17-hydroxycorticosteroids in peripheral blood which react with phenylhydrazine is 17-hydroxycorticosterone. These authors obtained blood samples from subjects with cardiac failure and hypertension. Whether such subjects have steroid patterns differing from those of normal subjects is not known. It is possible that in the Nelson-Samuels method a combination of low recovery of steroids from plasma and measurement of 17-hydroxysteroids other than 17-hydroxycorticosterone yields values approximating those of the fluorescent method.

Morris and Williams (4) recently reported the analysis of four steroids in human blood plasma: 11-dehydrocorticosterone, 2.0 to 8.5 μg.; corticosterone, 4.0 to 10.5 μg.; cortisone, 2.5 to 9.0 μg.; and 17-hydroxycorticosterone, 6.5 to 10.5 μg. per 100 ml. of plasma. Relatively high concentrations of 21-hydroxy-20-ketosteroids as determined by the Mader-Buck (17) reaction were observed in human plasma by Weichselbaum and Margraf (6, 11).

Bush and Sandberg (5) reported data which they suggest may be indicative of the presence of cortisone in human peripheral blood. Evidence for the presence of 11-dehydrocorticosterone has not been reported by investigators other than Morris and Williams.

Bush and Sandberg (5) resolved extracts of human blood by paper chromatography. Steroids were located and estimated semi-quantitatively by NaOH-induced yellow fluorescence. Corticosterone was detected in

only 4 of 14 samples. The lowest ratio of 17-hydroxycorticosterone to corticosterone was 4:1. In samples in which corticosterone was not detected, Bush and Sandberg calculated the ratio to be from >4.5:1 to >30:1. The spot test employed by these investigators may not be sufficiently sensitive to detect corticosterone unless the steroid is located in a small area of the paper chromatogram. The sensitivity of a spot test is limited by the area of spread of the steroid on paper, as well as by the total amount of the steroid. We are in agreement with Bush and Sandberg that the discrepancy between their results, those of Morris and Williams (4), and those of Sweat *et al.* (1) requires further investigation. A definitive statement cannot be made at present in regard to the concentration of corticosterone in human peripheral blood.

The proportion of B to F in blood is of some interest. As previously noted (Table 5), the ratios of B to F in adrenal venous blood of a patient undergoing an operation, and in peripheral blood obtained from the same patient before surgery and during surgery remained essentially the same. However, ratios of B to F in different subjects vary widely and do not appear to conform to any pattern. It is possible that the relative rate of synthesis of these two steroids may vary from subject to subject, or that relative rates of tissue degradation and intestinal and kidney excretion may influence the magnitude of the ratio.

SUMMARY

1. Human adrenal venous blood and peripheral blood have been analyzed for corticosterone and 17-hydroxycorticosterone by a fluorescence technique combined with a micro silica-gel column.

2. Human adrenal venous blood contains relatively high concentrations of both corticosterone and 17-hydroxycorticosterone. The adrenal venous blood from a patient with Cushing's disease contained 203 μg. of corticosterone and 880 μg. of 17-hydroxycorticosterone per 100 ml.

3. Human peripheral blood contains 17-hydroxycorticosterone and a second fluorescent steroid with chromatographic and fluorescent properties which suggest that it is corticosterone.

4. The average concentration of 17-hydroxycorticosterone in peripheral blood plasma of normal subjects is 10.8 μg. (±2.6, S.D.; ±0.57 S.E.) per 100 ml. The corticosterone-like compound is present in an average concentration of 4.3 μg. (±2.3, S.D.; ±0.50 S.E.) per 100 ml.

5. Plasma 17-hydroxycorticosterone is increased in concentration in patients with Cushing's disease.

6. The corticosterone-like substance was increased in concentration in the peripheral blood plasma of 1 patient with bilateral adrenal hyperplasia, but was within normal range in 2 patients with adrenal tumors.

UNIVERSITY OF MICHIGAN LIBRARIES

7. The administration of ACTH to normal subjects induces a two- to five-fold increase in the plasma concentration of both 17-hydroxycorticosterone and the corticosterone-like steroid.

8. The administration of ACTH to patients with Addison's disease does not result in a significant increase in the concentration of plasma fluorescent steroids.

Acknowledgment

The author is indebted to Drs. Katherine L. Sydnor, W. E. Abbott and W. M. Jefferies for collecting the samples of human adrenal venous blood; to Dr. Gordon L. Farrell for the phenylhydrazine analyses; and to Dr. George Sayers for his council and friendly criticisms throughout this investigation.

Addendum

Since the preparation of this manuscript, additional experimental data on peripheral blood adrenocorticosteroids have been accumulated.

1. *Adrenalectomized-oophorectomized patients* (Values in μg. of steroids per 100 ml. of plasma): Patient M.—F, 2.9; B-like, 1.8. Patient A.—F, 2.8; B-like, 1.6. Patient L.—F, 3.5; B-like 1.85. Blood samples for analysis were collected forty-eight to seventy-two hours after the last dose of oral steroids. No significant increases in steroid levels were observed in any of the patients two to four hours after the intravenous infusion of 40 units of ACTH. The observation that some steroids may be present in patients after adrenalectomy emphasizes the importance of the investigation of Graham (*Cancer* **6**: 149–152, 1953), who has found accessory adrenal cortex tissue at autopsy in 33 per cent of a group (100) of consecutive unselected cases.

2. *Adrenogenital syndrome.* Results of analysis of peripheral blood from 3 patients exhibiting clinical signs of adrenal insufficiency were as follows (post-ACTH values in parentheses): Patient K.—F, 12.0 (11.7); B-like, 2.8 (8.0). Patient J.—F, 11.5 (9.0); B-like, 11.0 (9.7). Patient B.—F, 7.8 (11.7); B-like, 7.0 (12.5). ACTH had little effect in increasing the quantities of peripheral blood steroids in these patients. These data confirm the observations of Grumbach *et al.* (*Bull. Johns Hopkins Hosp.* **96**: 116–125, 1955) and Kelley *et al.* (*Proc. Soc. Exper. Biol. & Med.* **81**: 611–614, 1952) on the effects of ACTH on blood steroid levels in their patients with the adrenogenital syndrome.

3. *Addison's disease.* The results of an analysis of peripheral blood from a pretherapy patient, not in a state of crisis, but exhibiting certain clinical signs of adrenal insufficiency were as follows: (post-ACTH values in parentheses): F, 2.8 (6.6); B-like, 0.43 (3.1). Our studies on patients with Addison's disease are consistent with those of Christy *et al.* (*J. Clin. Invest.* **34**: 899–906, 1955) and Eik-Nes *et al.* (*J. Clin. Invest.* **33**: 1502–1508, 1954; *J. Clin. Endocrinol. & Metab.* **15**: 13–21, 1955), who have observed that the administration of ACTH to patients with Addison's disease either does not increase the quantities of corticosteroids in peripheral blood or increases them to only a limited degree. Our observations are also consistent with those of Haydar *et al.* (*J. Clin. Endocrinol. & Metab.* **15**: 858, 1955), who have found normal quantities of 17-hydroxycorticosteroids in the urine of a group of patients with Addison's disease.

4. *Adrenal venous blood.* The recent studies of Hudson and Lombardo (*J. Clin. Endocrinol. & Metab.* **15**: 324–330, 1955) have added to knowledge in this field. In addition to compounds F and B, they have reported the presence of several trace substances

which may be steroids. Their quantitative data are in agreement with those of Sweat *et al.* (1) and Romanoff *et al.* (2).

5. *Normal peripheral blood.* Recent paper chromatographic analyses in our laboratory lend support to conclusions based on the silica-gel fluorescence technique. Ultraviolet-absorbing materials corresponding to the R_F of F and B have been observed in extracts from four 225-ml. samples of human plasma which were extracted, partitioned and chromatographed on 0.5-gram quantities of silica gel before chromatography on paper. Quantitative estimation by eye suggests that the quantities in the B spots are not over one third the quantities in the F spots. This ratio appears to be somewhat lower than that observed with the fluorescence method. Whether this quantitative difference is due to the spreading of the B spot in the presence of small quantities of fat-like material which accompanies B throughout the purification procedure or is due to the presence of small quantities of nonspecific material measured by the fluorescence procedure can be answered only by future refinements in methodology.

In addition to the spots in the F and B regions, three additional ultraviolet-absorbing spots were observed on the paper chromatograms: 1) A highly polar ultraviolet-absorbing spot which corresponded to that of Reichstein's substance E, and proved to be non-steroidal in nature, as spectrometric analysis demonstrated a 270-mμ absorption peak. 2) A spot which migrated at a rate corresponding to that of compound E. 3) A spot which represented a low polar compound, migrating at a rate similar to that of Reichstein's substance S and Kendall's compound A.

6. *Conjugated steroids of blood.* The question of the significance of conjugated cortical steroids in peripheral blood has recently been investigated by Bongiovanni and Eberlein (*Proc. Soc. Exper. Biol. & Med.* **89:** 281–285, 1955; **87:** 282–285, 1954) and Brown and Englert (*J. Clin. Invest.* **34:** 924, 1955). These authors have observed that 17-hydroxy-corticosteroids are released from blood plasma after hydrolysis with β-glucuronidase. Evidence at hand suggests that tetrahydro derivatives of 17-hydroxycorticosterone exist in the form of a glucuronide. Whether F exists in the form of a 21-OH glucuronide remains a question. In this laboratory, two 225-ml. samples of human blood plasma were extracted and hydrolyzed according to the procedure of Bongiovanni. The extract contained substances which reacted with phenylhydrazine but lacked ultraviolet-absorbing material in the F region of the paper chromatogram (sensitivity <1 μg. of steroid).

The foregoing clinical studies were carried out in collaboration with Dr. Harvey Kreiger, Department of Surgery and Dr. Sam Spector, Department of Pediatrics, Western Reserve University School of Medicine.

REFERENCES

1. Sweat, M. L.; Abbott, W. E.; Jefferies, W. M., and Bliss, E. L.: Adrenocortical steroids in human peripheral and adrenal venous blood as determined by fluorescence, *Fed. Proc.* **12:** 141, 1953.
2. Romanoff, E. B.; Hudson, P., and Pincus, G.: Isolation of hydrocortisone and corticosterone from human adrenal vein blood, *J. Clin. Endocrinol. & Metab.* **13:** 1546–1548, 1953.
3. Nelson, D. H., and Samuels, L. T.: A method for the determination of 17-hydroxycorticosteroids in blood: 17-hydroxycorticosterone in the peripheral circulation, *J. Clin. Endocrinol. & Metab.* **12:** 519–526, 1952.
4. Morris, C. J. O. R., and Williams, D. C.: The polarographic estimation of steroid hormones, *Biochem. J.* **54:** 470–475, 1953.

5. Bush, I. E., and Sandberg, A. A.: Adrenocortical hormones in human plasma, *J. Biol. Chem.* **205**: 783–793, 1953.
6. Weichselbaum, T. E.; Margraf, H. W., and Elman, R.: Quantitative determination of 17-hydroxycorticosteroids and 21-hydroxy-20-ketosteroid in the peripheral blood of humans before and after operative trauma, *Fed. Proc.* **12**: 287, 1953.
7. Kassenaar, A.; Molenaar, A.; Nijland, J., and Querido, A.: Estimation of 17-hydroxycorticosteroids in peripheral human blood, *J. Clin. Endocrinol. & Metab.* **14**: 746, 1954.
8. Bayliss, R. I. S., and Steinbeck, A. W.: A modified method for estimating 17-hydroxycorticosteroids in plasma, *Biochem. J.* **54**: 523–527, 1953.
9. Silber, R. H., and Porter, C. C.: The determination of 17,21-dihydroxy-20-ketosteroids in urine and plasma, *J. Biol. Chem.* **210**: 923–932, 1954.
10. Bondy, P. K., and Altrock, J. R.: Estimation of the rate of release of adrenal 17-hydroxycorticosteroids in the human being by the venous catheter technique, with a method for determining plasma 17-hydroxycorticosteroids, *J. Clin. Invest.* **32**: 703–710, 1953.
11. Weichselbaum, T. E., and Margraf, H. W.: Determination in plasma of free 17-hydroxy and 17-desoxy corticosteroids and their glucuronic-acid conjugates, *J. Clin. Endocrinol. & Metab.* **15**: 970–990, 1955.
12. Sweat, M. L.: Silica gel micro column for the chromatograghic resolution of cortical steroids, *Analyt. Chem.* **26**: 1964–1967, 1954.
13. Porter, C. C., and Silber, R. H.: A quantitative color reaction for cortisone and related 17,21-dihydroxy-20-ketosteroids, *J. Biol. Chem.* **185**: 201–207, 1950.
14. Sweat, M. L.: Sulfuric acid-induced fluorescence of corticosteroids, *Analyt. Chem.* **26**: 773–776, 1954.
15. Nelson, D. H.; Samuels, L. T.; Willardson, D. G., and Tyler, F. H.: The levels of 17-hydroxycorticosteroids in peripheral blood of human subjects, *J. Clin. Endocrinol.* **11**: 1021–1029, 1951.
16. Bliss, E. L.; Sandberg, A. A.; Nelson, D. H., and Eik-Nes, K.: The normal levels of 17-hydroxycorticosteroids in the peripheral blood of man, *J. Clin. Invest.* **32**: 818–823, 1953.
17. Mader, W. J., and Buck, R. R.: Colorimetric determination of cortisone and related ketol steroids, *Analyt. Chem.* **23**: 1748, 1951.

CHANGES IN URINARY 17-HYDROXYCORTICOSTEROID EXCRETION DURING STRESSFUL LIFE EXPERIENCES IN MAN*

BASIL S. HETZEL, M.D.,† WILLIAM W. SCHOTTSTAEDT, M.D.,† WILLIAM J. GRACE, M.D. AND HAROLD G. WOLFF, M.D.

with the technical assistance of ALBA PLESCIA, JOHN M. WHITTINGTON AND BARBARA GEOGHAN

The Departments of Medicine and Psychiatry, New York Hospital-Cornell Medical Center, New York, New York

IN OTHER reports from this laboratory, evidence of changes in general metabolism and thyroid function during stressful life experiences associated with diuresis has been presented (1, 2). In order to evaluate the role of the adrenal cortex in these changes, concurrent measurement of adrenocortical function was carried out.

There are few data concerning the participation of the human adrenal cortex in adaptations to stress in comparison with the wealth of data from animal experiments (3). There are reports of changes in urinary corticoid output as an indication of the level of circulating adrenal hormone following surgical trauma and burns (4, 5); but evidence based on eosinophil counts in peripheral blood has now been shown to be inadequate as an indication of the level of circulating adrenocortical hormone (6).

In this study determinations of 17-hydroxycorticosteroid excretion in the urine, using a technique modified from Glenn and Nelson (7), have been carried out before, during and after acute stressful life experiences in the laboratory and during the course of everyday activities. A preliminary communication on these data has already appeared (8).

The chemical nature of the compounds measured by this technique in human urine has recently been described (9). Small amounts of the free steroids, compound E (cortisone) and compound F (hydrocortisone), are measured but the main components are the biologically inactive metabolites of cortisone and hydrocortisone, "tetrahydro E" and "tetrahydro F", respectively, which are excreted in conjugated form as glucuronides.

Received for publication July 17, 1954.

* This work was supported in part by the Research and Development Division, Office of The Surgeon General, Department of the Army, under Contract No. DA-49-007-MD-524. Supported in part by a grant from the Commonwealth Fund.

† Commonwealth Fellow in Medicine.

The recently described salt-active hormone "electrocortin" (aldosterone) is not, however, measured by this technique (10).

METHODS

The methods used in the study of stressful life experiences in the laboratory have been described elsewhere (1). Subjects from the New York Hospital were studied by means of a full medical history, physical examination and appropriate laboratory investigation. Evidence of areas of significant interpersonal conflict was particularly sought by study of the life history, background and attitudes of the subject.

In brief, the laboratory studies consisted of a preliminary period during which the patient was diverted and maintained as far as possible in a relatively tranquil state. After approximately an hour, a discussion was initiated which, on the basis of previous study, was designed to be stressful to the subject. Finally, after this interview (lasting about an hour) an attempt was made to re-establish a feeling of security in the patient. Observations during which no stimulus was introduced were also carried out. These observations were made in the postabsorptive state, with no fluid intake from the previous evening.[1]

Specimens of urine were collected on arrival at the laboratory and at the end of each of the three observation periods. The volume was measured and the excretion rate expressed in cc./minute. Adequacy of collection was checked by determination of creatinine, using a modification of the method of Bonsnes and Taussky (11). Determinations of urinary total nitrogen (Koch McMeekin), sodium, and potassium (Barclay flame photometer) were carried out in addition to the assay of 17-hydroxycorticosteroids.

Similar determinations were carried out on urine specimens collected during the day by 3 healthy young physicians. These physicians kept a diary of their everyday activities and variations in feeling state. The data were correlated with corticoid output. A record of food and fluid intake was also kept. These data are to be reported elsewhere (12, 13).

The reagents used were those described by Glenn and Nelson (7), except that penicillin was not added to the β-glucuronidase. Fifteen-cc. aliquots of urine were generally used. The colorimetry was carried out with a Coleman Universal spectrophotometer.

The standard curve was checked periodically and found to be steady. Recoveries of free hydrocortisone added to urine and put through the whole procedure remained steady at 60 per cent. Determinations were carried out in duplicate, the results being expressed in micrograms (gamma) per minute. The standard deviation of a mean of duplicates over the range of values found was 0.84 gamma/minute, which meant that a change of ± 1.7 gamma/minute was significant at the 95 per cent level. All the determinations in any one series of observations were performed at the same time with the same batch of Florisil, so that the changes observed are believed to reflect changes in corticoid excretion.

RESULTS

The data may be conveniently divided into two categories: 1) oberva-

[1] The original stressful life experiences and the recollections thereof are not identical, although they have much in common. The subject, in the presence of the physician, not only recalls the situation and experiences the conflict and other reaction; but, through the inquiries and participation of the physician, he has these reactions revived and even intensified during the exchange.

tions during the morning hours, and 2) observations throughout twenty-four hours over periods of four to six hours.

1. *Observations during the morning hours*

These data are summarized in Table 1. Observations were made on 11 different subjects, a few of whom will now be considered in detail.

Subject I. **M.L.,** a 21-year-old single male of Italian extraction with a chief complaint of loss of weight, came to the laboratory for determinations of his gaseous exchange, serum protein-bound iodine (PBI) and urinary nitrogen excretion, as a check on his thyroid function. He was pleased to have the test, came in, and remained relatively tranquil for a three-hour period of observation during which the various determinations were made at approximately hourly intervals. In Figure 1 are shown the results of the determinations of 17-hydroxycorticosteroid excretion, nitrogen excretion and urine flow. It will be seen that little change from the initial level of 3.5 gamma/minute for 17-hydroxycorticosteroid excretion was observed, although there was a fall in total nitrogen excretion and urine volume. In addition, the serum PBI remained steady at levels between 4.1 and 4.4 gamma per 100 cc., and his gaseous exchange also remained steady.

Subject II. **E.B.** was a 57-year-old female with symptoms of nervousness, tremor, fever and loss of weight suggestive of Graves' disease, who was also fearful of having cancer of the breast or heart disease. On coming to the laboratory for study of her thyroid function, she was noted to be apprehensive, remarking, "I'm not going to have a cardiograph, am I?" The initial level of 17-hydroxycorticosteroid excretion was 8.3 gamma/minute, total nitrogen 5.2 mg./minute, urine volume 0.99 cc./minute, sodium 173 μEq./minute, and potassium 29 μEq./minute (Fig. 2).

An attempt was made to alleviate her initial anxiety by a generally reassuring attitude during the first hour. Over this period 17-hydroxycorticosteroid excretion was 6.3 gamma/minute, nitrogen excretion 4.7 mg./minute, urine flow 0.89 cc./minute, sodium

TABLE 1. URINARY 17-HYDROXYCORTICOSTEROID EXCRETION (GAMMA/MINUTE) DURING THE MORNING HOURS

No.	Subject			Diagnosis	On Arrival*	1st Hour	2nd Hour	3rd Hour	Δ**
	Name	Sex	Age						
				(a) *Observations during periods of relative tranquillity*					
1	M.L.	M	21	Gastric hypofunction	3.0	3.9	3.0	2.7	−0.9
2	R.S.	F	45	Migraine	1.9	2.2	1.4	1.9	−0.8
				(b) *Observations during stressful life experiences*					
1	E.B. (1)	F	57	Neurasthenia	8.3	6.3	*8.3*	5.3	+2.0
2	E.B. (2)	F	57	Neurasthenia	6.7	6.0	*7.1*	5.8	+1.1
3	W.G.	M	37	Healthy	2.8	—	*8.0*	—	+5.2
4	K.M.	F	38	Neurasthenia	3.1	*4.3*	3.4	3.5	+1.2
5	A.T.	F	50	Neurasthenia	1.1	*2.3*	*1.8*	1.4	+1.2
6	I.G.	M	40	Essential hypertension	4.4	*3.5*	3.7	1.6	−0.9
7	C.B.	F	37	Anovular bleeding	3.7	4.6	*5.2*	3.2	−1.4
8	M.B.	F	28	Hypothyroid (rec'g thyroid)	2.7	*5.5*	2.1	0.8	+2.8
9	H.P.	F	53	Hypothyroid (rec'g thyroid)	3.4	4.3	*6.0*	*9.6*	+1.7
10	J.A.	M	60	Hypothyroid (untreated)	1.4	*1.6*	*1.2*	0.7	+0.2

* Between 9:00 and 9:30 a.m.

** Difference between period of stress (italic) and preceding period.

excretion 162 μEq./minute and potassium excretion 28 μEq./minute. In the second hour her fears about her heart and a previous lump in her breast were discussed with her. In addition, she told of the loss of her only son, a Navy pilot, in a crash five years before. She vividly described the foreboding she had had about it. She became much more apprehensive over this period. 17-Hydroxycorticosteroid excretion rose to 8.3 gamma/minute, nitrogen excretion rose to 8.5 mg./minute and urine flow to 2.08 cc./minute; the sodium excretion was 191 μEq./minute and potassium excretion was 46 μEq./minute. Following the discussion she was reassured as to the absence of any serious disease; she accepted this well, was considerably relieved, and settled down to reading a news magazine. Over this period the 17-hydroxycorticosteroid excretion dropped to 5.3 gamma/minute, nitrogen excretion to 3.4 mg./minute, urine flow to 0.61 cc./minute, sodium excretion to 118 μEq./minute and potassium excretion to 30 μEq./minute. Gaseous exchange determinations showed a significant increase in oxygen consumption with a fall in respiratory quotient coincident with the increase in total nitrogen and 17-hydroxycorticosteroid excretion.

The study was repeated in the same subject three months later (Fig. 3). On this occasion she was again very apprehensive on coming to the laboratory. Initial levels for 17-hydroxycorticosteroid excretion were 6.7 gamma/minute, nitrogen excretion 7.4 mg./minute, urine flow 1.21 cc./minute, sodium excretion 172 μEq./minute and potassium excretion 22 μEq./minute. A slight fall occurred in all these values (except potassium) during the first period of observation.

During the second hour, an attempt was made to discuss relatively neutral material, such as recreational interests, with the subject. Although this was not entirely successful, the patient was less apprehensive than on the previous occasion. There was only a slight increase in 17-hydroxycorticosteroid and nitrogen excretion, though significant increases in urine flow and sodium excretion were observed. All values fell during the third hour, when the patient was reading and sewing.

Subject III. **M.B.** was a 28-year-old single woman of subnormal intelligence, with a history of hypothyroidism from infancy. At the time the study (Fig. 4) was carried out she was being adequately maintained on 90 mg. of desiccated thyroid daily. She was asked to come to the laboratory for a test but was unaware of its connection with an investigation of changes in thyroid function associated with exposure to low temperature.

Following collection of an initial specimen of urine and blood, she was told that after an hour she was to be placed in a cold room for about an hour. Her demeanor immediately indicated considerable fear and dread of this possibility. She was left alone for an hour and not reassured. Over this period 17-hydroxycorticosteroid output was 5.5 gamma/minute (having increased from 3.7 gamma/minute), nitrogen excretion rose slightly to 6.0 mg./minute from 4.9 mg./minute, and there was a marked increase in urine flow from 0.50 to 1.2 cc./minute with little change in sodium or potassium excretion.

On placing her in the cold room she soon realized that she was not nearly so uncomfortable as she had anticipated and lost her fears. Over this time 17-hydroxycorticosteroid output dropped to 2.1 gamma/minute, total nitrogen excretion was 4.5 mg./minute and urine volume 0.64 cc./minute. There was a further fall in all these levels after another hour during which she was out of the cold room, reading and in a relatively tranquil state. The serum PBI level did not vary significantly over the whole period of observation—the mean being 4.0 gamma per 100 cc.

Subject H.P. Similar changes were observed in another hypothyroid subject (H.P.) in association with dread about the possibility of cardiac catheterization being performed. This subject was not clinically euthyroid although she was being maintained on

120 mg. of desiccated thyroid, and her serum cholesterol level had fallen from 376 mg. to 218 mg. per 100 cc. There was a rise in 17-hydroxycorticosteroid output from 3.4 to 9.6 gamma/minute (Table 1).

Comment. These studies demonstrate levels of urinary 17 hydroxycorticosteroid excretion in the range 5.5–9.6 gamma/minute during states of dread and apprehension in 3 subjects (E.B., M.B. and H.P.) when exposed to certain threatening stimuli. Observations on M.B., H.P. and 2 other subjects, M.L. and R.S., during periods of relative tranquillity when they felt secure and free from threats showed levels in the range 1.4–4.6 gamma/minute.

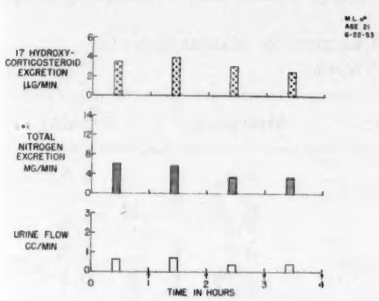

FIG. 1

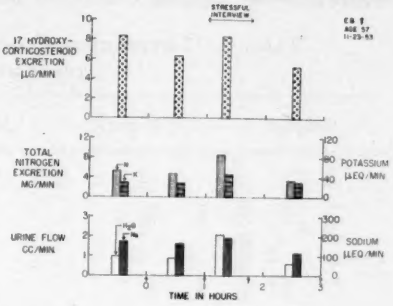

FIG. 2

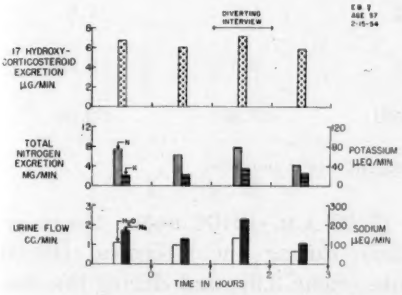

FIG. 3

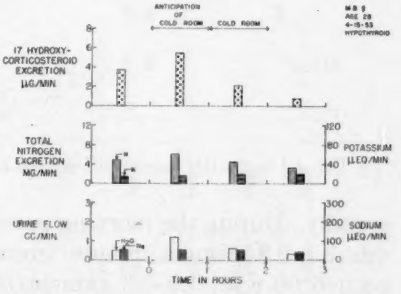

FIG. 4

FIG. 1. Steady level of urinary 17-hydroxycorticosteroid excretion during a morning period of relative tranquillity.

FIG. 2. Changes in urinary 17-hydroxycorticosteroid, total nitrogen and electrolyte excretion in association with a stressful interview.

FIG. 3. Smaller changes in urinary 17-hydroxycorticosteroid and total nitrogen excretion in association with a diverting interview in the subject represented in Figure 2.

FIG. 4. Changes in urinary 17-hydroxycorticosteroid excretion in association with unpleasant anticipation of a period in the cold room.

However, in 3 other subjects (A.T., K.M. and I.G.) (Table 1), only slight increases from levels during tranquillity accompanied periods of similar dread and apprehension associated with marked diuresis (Table 3). These studies will be discussed subsequently.

2. *Observations throughout twenty-four hours*

Observations on 3 healthy research physicians during the course of their everyday activities are summarized in Table 2, according to the time of day. These subjects were not fasting during this period of study.

The data indicate a range of 1.9–3.6 gamma/minute (mean 2.6) for night periods (11:00 P.M.–7:00 A.M.) during which the 3 subjects slept

TABLE 2. 17-HYDROXYCORTICOSTEROID EXCRETION (GAMMA/MINUTE) THROUGHOUT 24 HOURS

Subject	Night	Morning	Afternoon	Evening
M.T.	2.7	3.1	5.3	0.8
W.S. 1.	2.2	6.5	2.2	1.3
2.	1.7	6.8	3.6	1.0
3.	—	—	1.9	0.7
B.H. 1.	2.8	3.4	5.6	2.5
2.	1.9	4.6	4.2	2.0
3.	3.6	4.2	4.7	1.6
Mean	*2.5*	*4.8*	*4.6*	*1.65*
*P**	—	*<0.001*	*<0.001*	*<0.05*

* Using *t* test with paired differences comparing night periods.

soundly. During the morning periods (7:00 A.M.–12:00 noon) the range was 3.1–6.8 gamma/minute (mean 3.6); during the afternoon (12:00 noon–6:00 P.M.) 1.9–5.6 gamma/minute (mean 3.9); and during the evening (6:00 P.M.–11:00 P.M.) 0.7–2.0 gamma/minute (mean 1.4). Statistical analysis demonstrated that levels during the morning and afternoon were significantly higher, and those during the evening significantly lower than levels at night (Table 2). Examples will now be considered in detail.

Subject I. **W.S.** was a 34-year-old research physician who soon after arrival at his office on April 28, 1953 received a telephone call from one of his patients which indicated that her state of depression had become so much more severe that she was a serious suicidal risk. He felt very agitated, dreading what might happen, very uncertain as to what he should do, but actively considering various alternatives. He remained preoccupied with this problem most of the morning. Over this period, 17-hydroxycorti-

costeroid excretion was 6.8 gamma/minute, having been only 1.7 gamma/minute during the night, nitrogen excretion was 11.1 mg./minute, urine flow 2.46 cc./minute (average morning excretion 0.92 cc./minute), sodium excretion 303 μEq./minute (average morning excretion 139 μEq./minute), and potassium excretion 86 μEq./minute (average morning excretion 66 μEq./minute) (Fig. 5). After lunch he was more calm. During the afternoon 17-hydroxycorticosteroid excretion was 3.6 gamma/minute and nitrogen excretion 6.4 mg./minute.

Subject II. **B.H.** was a 31-year-old research physician who was in a relatively tranquil state during the morning hours, at which time his 17-hydroxycorticosteroid excretion was 3.4 gamma/minute, nitrogen excretion 6.0 mg./minute, urine flow 0.51 cc./minute, sodium excretion 136 μEq./minute, and potassium excretion 41 μEq./minute (Fig. 6). During the afternoon he met a senior colleague with whom he discussed some of his research problems and his ideas for their solution. The senior colleague was sympathetic

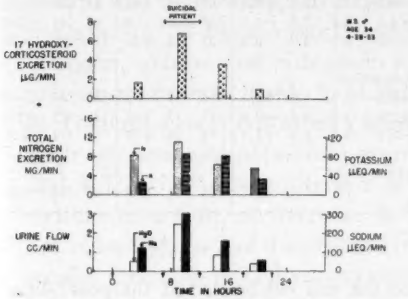

FIG. 5

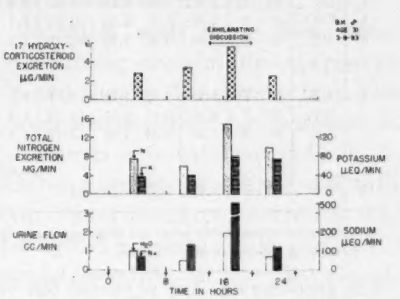

FIG. 6

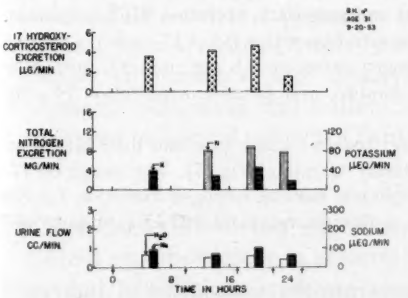

FIG. 7

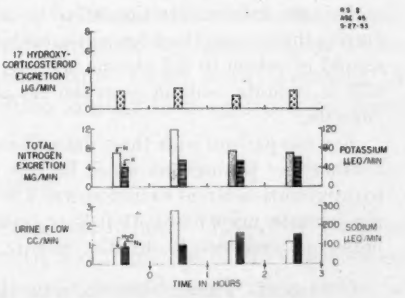

FIG. 8

FIG. 5. Changes in urinary 17-hydroxycorticosteroid, total nitrogen and electrolyte excretion in association with a stressful situation.

FIG. 6. Changes in urinary 17-hydroxycorticosteroid, total nitrogen and electrolyte excretion in association with an exhilarating experience.

FIG. 7. Smaller changes in urinary 17-hydroxycorticosteroid, total nitrogen and electrolyte excretion on another day in the subject represented in Figure 6.

FIG. 8. Absence of significant change in 17-hydroxycorticosteroid excretion in association with diuresis during a period of migraine headache.

TABLE 3. CHANGES IN URINE FLOW AND 17-HYDROXYCORTICOSTEROID, TOTAL NITROGEN, SODIUM AND POTASSIUM EXCRETION DURING STRESSFUL LIFE EXPERIENCES

No.	Subjects				Urine flow (cc./min.)			17-OH-corticoid (gamma/min.)			Total nitrogen (mg./min.)			Potassium μEq./min.			Sodium μEq./min.		
	Name	Age	Sex	S.A.	Before	After	Δ*	Before	After	Δ	Before	After	Δ	Before	After	Δ	Before	After	Δ
								(a) *Euthyroid subjects*											
1	E.B.	57	F	1.63	0.89	2.08	+1.19	6.3	8.3	+2.0	4.7	8.5	+3.8	28	46	+18	162	191	+29
2	W.G.	36	M	1.92	0.71	1.72	+1.01	2.8	8.0	+5.2	5.7	12.9	+7.2	61	88	+27	147	363	+216
3	K.M.	38	F	1.45	1.19	4.74	+3.55	3.1	4.3	+1.2	7.1	10.0	+2.9	27	49	+22	93	180	+87
4	W.S.†	35	M	1.83	0.54	2.46	+1.92	1.7	6.8	+5.1	8.3	11.1	+2.8	26	86	+60	123	303	+180
5	B.H.†	31	M	2.24	0.51	1.97	+1.46	3.4	5.6	+2.2	6.0	14.8	+8.8	41	80	+39	136	507	+371
6	M.T.†	31	M	2.08	0.85	1.24	+0.39	3.1	5.3	+2.2	8.8	11.8	+3.0	39	67	+28	177	289	+112
7	I.G.	40	M	1.97	0.78	2.00	+1.22	4.4	3.5	−0.9	10.3	16.0	+5.7	64	24	−40	81	298	+217
8	C.B.	37	F	1.66	2.7	3.85	+1.15	3.7	3.2	−0.5	9.6	8.5	−1.1	118	108	−9	81	244	+163
9	A.T.	50	F	1.40	0.82	3.00	+2.18	1.1	1.8	+0.7	6.3	6.3	0.0	55	108	+53	138	228	+90
								(b) *Hypothyroid subjects*											
1	M.B.	28	F	1.55	0.50	1.21	+0.71	2.7	5.5	+2.8	4.9	6.0	+1.1	15	17	+2	55	54	−1
2	H.P.	53	F	1.82	0.67	2.41	+1.74	3.4	6.0	+2.6	4.5	6.5	+2.0	55	72	+17	148	422	+274
3	J.A.	60	M	1.89	0.81	1.93	+1.12	1.4	1.2	−0.2	4.8	5.9	+1.1	64	93	+29	99	174	+75
								(c) *Adrenalectomized subject*											
1	R.H.	40	F	1.69	0.47	0.78	+0.31	—	—	—	7.1	11.3	+4.2	24	34	+10	87	133	+46

* Difference between levels before and after stressful stimulus.
† Periods of observation were 4–6 hours.

and encouraging. The physician felt very excited and exhilarated at the possibilities opened up by the discussion. During the period of five hours, 17-hydroxycorticosteroid excretion was 5.6 gamma/minute, nitrogen excretion 14.8 mg./minute, urine flow 1.97 cc./minute, sodium excretion 507 μEq./minute and potassium excretion 80 μEq./minute. During the evening these feelings subsided in association with a fall in 17-hydroxycorticosteroid excretion to 2.5 gamma/minute, nitrogen excretion 9.8 mg./minute, urine flow 0.73 cc./minute, sodium excretion 123 μEq./minute, and potassium excretion 74 μEq./minute.

For comparison with these data, the subject took a similar food and fluid intake on another day throughout which he was relatively tranquil (Fig. 7). The range of 17-hydroxycorticosteroid excretion was 1.9–4.6 gamma/minute, nitrogen excretion 7.8–8.9 mg./minute, urine flow 0.41–0.61 cc./minute, sodium excretion 69–102 μEq./minute and potassium excretion 19–46 μEq./minute.

Comment. These observations demonstrate the association of increased urinary 17-hydroxycorticosteroid output with responses to stimuli similar to those described in the short-term studies. They suggest that the well marked diurnal variations in 17-hydroxycorticosteroid excretion may be due to such responses.

Relation of increases in 17-hydroxycorticosteroid excretion to other urinary constituents

It will have been noted from Figures 2, 4, 5 and 6 that increases in 17-hydroxycorticosteroid excretion in association with stressful life experi-

ences were coincident with increases in urine flow, and in total nitrogen, sodium and potassium excretion. The question arises (14) as to whether the increase in 17-hydroxycorticosteroid excretion is simply dependent on an increase in urine flow. That this is not the case is clearly demonstrated by the study shown in Figure 8.

Subject R.S. (Fig. 8) was a 45-year-old woman who came to the laboratory with a severe migraine headache. She was made as comfortable as possible. During the first hour of observation she had a spontaneous diuresis with an increase of urine flow to 2.78 cc./minute from an initial level of 0.85 cc./minute. Associated with this increase there was an increase in total nitrogen excretion but no significant change in 17-hydroxycorticosteroid output and little change in sodium and potassium excretion.

Data on 17-hydroxycorticosteroid excretion with urine flow, total nitrogen, sodium and potassium excretion during stressful life experiences are summarized in Table 3. It will be noted that in 6 instances increases in 17-hydroxycorticosteroid excretion were accompanied by increases in both nitrogen and potassium excretion, whereas in 3 instances (I.G., C.B. and A.T.) when either nitrogen or potassium excretion was increased with sodium excretion, an increase in 17-hydroxycorticosteroid excretion was not observed. In 2 of 3 instances in hypothyroid subjects (M.B. and H.P.) similar increases in 17-hydroxycorticosteroid excretion occurred with smaller increases in nitrogen and potassium excretion (3). In the other instance (J.A.) no increase was observed; this patient was untreated and had a serum PBI level of 1.6 gamma per cent in association with the classic clinical picture of myxedema. In 1 totally adrenalectomized subject maintained on cortisone and desoxycorticosterone there were increases in nitrogen, potassium and sodium excretion similar in magnitude to those observed in normal subjects (Table 3).

DISCUSSION

The data indicate that significant increases in urinary 17-hydroxycorticosteroid excretion occur in association with certain stressful life experiences in man. Since these increases are not solely dependent on an increase in urine flow, it is assumed that they do reflect changes in hormone output from the adrenal cortex and are not related to increased glomerular filtration rate. These increases have been found to be associated with feeling states similar to those noted in relation to general metabolic changes, both in acute experiments (8) and in longer term studies (13). In particular, feeling states described by the subjects as apprehension or dread and exhilaration or excitement have been found to be associated with increases in 17-hydroxycorticosteroid excretion.

These findings are in contrast to those of Thorn *et al.* (6), who found no

change in 17-hydroxycorticosteroid excretion associated with exposure of human subjects to low temperature and anoxia. It is of interest that the observations on Subject M.B. in this study indicate that the fearful anticipation of exposure to low temperature was a far more potent stimulus to adrenocortical function than actual presence in the cold room.

As might be anticipated, the variation observed over 1-hour periods of observation (0.8–9.6 gamma/minute or 1.2–13.8 mg./day) was greater than that observed for 4-hour to 6-hour periods (0.7–6.8 gamma/minute or 1.0–9.8 mg./day). This latter range is similar to that reported by Sandberg *et al.* (0.4–7 mg./day) for 8-hour to 12-hour periods of observation, using the same technique (15). Sandberg *et al.* also found a diurnal variation in 17-hydroxycorticosteroid excretion similar to that found in this study, and Pincus has reported a similar diurnal variation in 17-ketosteroid excretion (16). It would seem possible from the evidence presented here that the reason for this variation is exposure to stressful stimuli during the active period of the day in these particular subjects. Such factors may also account for the wide normal range of variation over 24-hour periods observed with this technique and with the more specific biologic assays (17, 18).

As noted previously, increases in 17-hydroxycorticosteroid output were accompanied by increased total nitrogen, sodium and potassium excretion in euthyroid subjects. In 1 case there was also demonstrated an increase in oxygen consumption and a decrease in the nonprotein respiratory quotient which has been shown elsewhere (2) to accompany the increase in total nitrogen excretion. In general, therefore, the changes are associated with presumptive evidence of protein and fat catabolism characteristic of the metabolic response to other types of injury in man, such as operative trauma (19) and infections (20). However, when the catabolic response is less, as in hypothyroid subjects undergoing treatment with thyroid (2, 12), increases in 17-hydroxycorticosteroid excretion are unaffected. Observations in 1 adrenalectomized patient revealed increases in nitrogen, sodium and potassium excretion similar to those observed in normal subjects during stressful life experiences. These findings suggest that the adrenal cortex is not involved in a primary causative or regulatory role in the catabolic response under the conditions of stressful life experiences in man. This is consistent with the "permissive" role of the adrenal cortex, which has been demonstrated in relation to the catabolic response following fractures (21) and following formalin injections (22) in animals. On the contrary, the regulation of the catabolic response associated with stressful life experiences in man may depend on the thyroid (2, 12) and this may also be true for the catabolic response following burns in animals (23–25).

SUMMARY

1. Urinary 17-hydroxycorticosteroid excretion has been measured before, during and after certain acute stressful life experiences in man associated with diuresis.

2. a) During such experiences, 17-hydroxycorticosteroid excretion was elevated in some subjects.

b) In the euthyroid subjects these elevated levels were associated with increases in total nitrogen, sodium and potassium excretion. In 2 hypothyroid subjects maintained on thyroid therapy, similar increases in 17-hydroxycorticosteroid excretion were accompanied by smaller changes in nitrogen and potassium excretion. In 1 adrenalectomized subject maintained on cortisone and desoxycorticosterone, increases in total nitrogen, potassium and sodium excretion similar to those in normal subjects were observed.

3. A series of observations throughout the day in 3 euthyroid subjects showed a well marked diurnal variation.

4. These findings suggest that the human adrenal cortex participates in stressful adaptations, and are consistent with the "permissive" role of the adrenal cortex in nitrogen metabolism.

Acknowledgments

We are indebted to Dr. C. D. West and Miss S. di Mauro of the Sloan-Kettering Institute, for technical advice; and to Dr. E. Shorr and Dr. E. Livingstone for cooperation in carrying out the study on the adrenalectomized subject.

REFERENCES

1. Hetzel, B. S.; de la Haba, D. S., and Hinkle, L. E., Jr.: Rapid changes in plasma PBI in euthyroid and hyperthyroid subjects, *Tr. Am. Goiter A.* 1952, p. 242.
2. Hetzel, B. S.; Schottstaedt, W. W., and Grace, W. J.: Evidence of regulatory role of thyroid in the excretion of nitrogen during stressful life experiences in man, (abstract) *J. Clin. Invest.* **33**: 941, 1954.
3. Selye, H.: The general adaptation syndrome and the diseases of adaptation, *J. Clin. Endocrinol.* **6**: 117, 1946.
4. Browne, J. S. L.; Johnson, L. G.; Schenker, V., and Venning, E. H.: Protein metabolism in acute and chronic disease and the relation of protein metabolism to the excretion of glucocorticoids, *in* Proceedings of the First ACTH Conference, ed. by John R. Mote. Philadelphia, The Blakiston Company, 1950.
5. Thorn, G. W.; Jenkins, D. and Laidlaw, J. C.: The adrenal response to stress in man, *Recent Progr. Hormone Res.* **8**: 171, 1953.
6. Sandberg, A. A.; Nelson, D. H.; Palmer, J. G.; Samuels, L. T., and Tyler, F. H.: The effects of epinephrine on the metabolism of 17-hydroxycorticosteroids in the human, *J. Clin. Endocrinol. & Metab.* **13**: 629, 1953.
7. Glenn, E. M., and Nelson, D. H.: Chemical method for the determination of 17-hydroxycorticosteroids and 17-ketosteroids in urine following hydrolysis with β-glucuronidase, *J. Clin. Endocrinol. & Metab.* **13**: 911, 1953.

8. HETZEL, B. S.; SCHOTTSTAEDT, W. W.; GRACE, W. J., and WOLFF, H. G.: Changes in urinary 17-hydroxycorticosteroid excretion during stressful life experiences in man, (abstract) *J. Clin. Endocrinol. & Metab.* **14:** 805, 1954.
9. COPE, C. L., and HURLOCK, B.: The estimation of some adrenocortical products in urine, *in* Determination of Adrenocortical Steroids and Their Metabolites. Memoirs of the Society for Endocrinology, No. 2. London, Dennis Dobson Ltd., 1953. p. 25.
10. SIMPSON, S., and TAIT, J. F.: Physico-chemical methods of detection of a previously unidentified adrenal hormone, *Ibid.*, p. 9.
11. BONSNES, R., and TAUSSKY, H.: On the colorimetric determination of creatinine by the Jaffe reaction, *J. Biol. Chem.* **158:** 581, 1945.
12. HETZEL, B. S.; SCHOTTSTAEDT, W. W.; GRACE, W. J., and WOLFF, H. G.: Changes in urinary nitrogen and electrolyte excretion during stressful life experiences and their relation to thyroid function (in preparation).
13. HETZEL, B. S.; SCHOTTSTAEDT, W. W., and GRACE, W. J.: Variations in nitrogen excretion during the day and their relation to stressful life experience (in preparation).
14. MARKS, L. J., and LEAF, A.: The relationship of the renal excretion of adrenal corticoids to variations in renal hemodynamics, *J. Clin. Invest.*, **32:** 813, 1953.
15. SANDBERG, A. A.; NELSON, D. H.; GLENN, E. M.; TYLER, F. H., and SAMUELS, L. T.: 17-Hydroxycorticosteroids and 17-ketosteroids in urine of human subjects: clinical application of a method employing β-glucuronidase hydrolysis, *J. Clin. Endocrinol. & Metab.* **13:** 1445, 1953.
16. PINCUS, G.: A diurnal rhythm in excretion of urinary ketosteroids by young men, *J. Clin. Endocrinol.* **3:** 195, 1943.
17. VENNING, E. H., and KAZMIN, V.: Excretion of urinary corticoids and 17-ketosteroids in the normal individual, *Endocrinology* **39:** 131, 1946.
18. HETZEL, B. S.; HINE, D. C., and STENHOUSE, N. S.: Adrenal cortical function in hypertension, *J. Clin. Endocrinol. & Metab.* **12:** 296, 1952.
19. MOORE, F. C.: The Metabolic Response to Surgery. A monograph in American Lecture Series. Springfield, Ill., Charles C Thomas, Publisher, 1950.
20. GROSSMAN, C. M.; SAPPINGTON, T. S.; BURROWS, B. A.; LAVIETES, P. H., and PETERS, J. P.: Nitrogen metabolism in acute infections, *J. Clin. Invest.* **24:** 523, 1945.
21. INGLE, D. J.; WARD, E. O., and KUIZENGA, M. H.: The relationship of the adrenal glands to changes in urinary nonprotein nitrogen following multiple fractures in the force fed rat, *Am. J. Physiol.* **149:** 510, 1947.
22. ENGEL, F. L.: On the nature of the interdependence of the adrenal cortex, nonspecific stress and nutrition in the regulation of nitrogen metabolism, *Endocrinology* **50:** 462, 1952.
23. GRIBBLE, M. DE G., and PETERS, R. A.: Influence of thyroidectomy on post-burn nitrogen loss in rats, *Quart. J. Exper. Physiol.* **36:** 119, 1951.
24. SELLERS, E. A.; YOU, S. S., and YOU, R. W.: The influence of adrenal cortex and thyroid on the loss of nitrogen in urine after experimental burns, *Endocrinology* **47:** 148, 1950.
25. WASE, A. W.; EICHEL, H. J., and REPPLINGER, E.: Relation of thyroid status to nitrogen excretion following exposure to thermal radiation, *Proc. Soc. Exper. Biol. & Med.* **84:** 152, 1953.

ADRENOCORTICAL ACTIVITY IN THE PREOPERATIVE PERIOD

CURT FRANKSSON, M.D. AND CARL A. GEMZELL, M.D.

The Department of Surgery and the Department of Obstetrics and Gynecology, Karolinska Hospital and Konung Gustaf V's Forskningsinstitut, Stockholm, Sweden

IN a previous paper it was reported that in the preoperative period the plasma levels of adrenocortical steroids in some patients were remarkably high (1). The eosinophil count has also been shown to diminish with the approach of surgery (2). This evidence of increased activity of the adrenal cortex is difficult to explain, since there was no treatment in the preoperative period. However, it is well known that patients who are going to be operated upon are more or less under psychic tension which may increase as the day of operation draws near, suggesting that the preoperative increase of adrenocortical activity may be due to emotional stimuli.

This paper is concerned with the level of plasma corticosteroids in patients during the preoperative period. The plasma levels of the same patients one and two days earlier were used as controls.

MATERIAL AND METHODS

Thirty-three patients in the surgical department of the Karolinska Hospital were selected at random. The average age was 56 years (range, 26 to 75 years). Eighteen were men and 15 were women. All endocrine cases were excluded. The patients were informed about the time of the operation at least one day in advance. The first blood sample was taken two days preoperatively, the second sample one day before, and the third the morning of the operation. The blood samples were taken between 7 and 8 A.M.

The level of 17-hydroxycorticosteroids in blood was determined according to the method of Nelson and Samuels (3). Approximately 10 ml. of plasma was extracted with chloroform. Chromatography was carried out on magnesium-silicate-Celite columns. Correction for background absorption was made by using the absorption factor of Allen (4). The concentration of 17-hydroxycorticosteroids in blood was expressed in μg. per 100 ml. of plasma. The normal concentration is considered to be 4–10 μg., with a mean value of 6.6 ± 0.9 μg. per 100 ml. of plasma.

Received for publication December 27, 1954.

RESULTS

The average plasma level of 17-hydroxycorticosteroids on the day of operation was 14.51 ± 1.46 μg. per 100 ml. of plasma. The average plasma level two days before the operation was 5.53 ± 0.52 μg. and one day before, 8.55 ± 1.02 μg. The difference between the plasma level on the day of

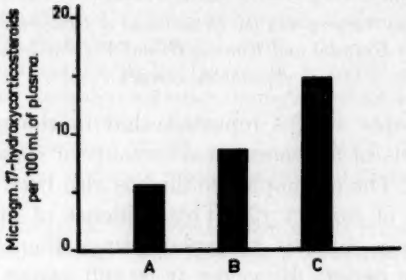

FIG. 1. Average plasma level of 17-hydroxycorticosteroids in 33 patients on the day of operation (C) and the two preceding days (A and B).

operation and the plasma levels on one or the other of the earlier days is highly significant ($p < 0.001$) (Table 1 and Fig. 1).

Of the 33 patients investigated, 26 showed a higher plasma level on the day of operation than on any of the two preceding days.

On the day of operation no difference was found in the plasma levels between men and women or between young and old patients.

DISCUSSION

Under normal conditions the activity of the adrenal cortex shows some diurnal variations. The activity is comparatively high during the first part of the day and decreases to a minimum late in the evening (5, 6, 7). These variations, however, are relatively small.

Following surgery or a traumatic incident there is increased adrenocortical activity. Postoperatively the plasma levels of cortical steroids follow a certain pattern, seemingly independent of the type of operation and the age and sex of the patient (1).

This study indicates that in the preoperative period, stimulation of the adrenal cortex occurs. Since the patients were not influenced by other factors which possibly could activate the adrenal cortex, it seems most likely that it is the emotional state which causes the observed stimulation.

Of the 33 patients investigated, 26 showed an increased plasma level of corticosteroids prior to the operation. In some cases the increase was pronounced, in others it was insignificant. No correlation was found between

TABLE 1

Name	Age	Diagnosis	Plasma levels of 17-hydroxycorticosteroids (μg./100 ml.)		
			Day 1	Day 2	Day 3 (operation)
BR	52	Testicular hydrocele	2.0	4.2	7.1
VA	73	Inguinal hernia	2.3	10.7	16.7
PN	52	Inguinal hernia	3.4	9.5	12.7
BE	44	Hemorrhoids	8.7	7.9	14.6
CM	52	Cholelithiasis	3.6	11.4	8.6
US	45	Cholelithiasis	3.5	8.5	8.5
AI	35	Cholelithiasis	2.4	2.0	7.0
BN	53	Cholelithiasis	2.0	2.0	5.0
LS	23	Cholelithiasis	2.0	2.0	25.4
DS	29	Nephrolithiasis	6.7	2.0	10.8
JO	67	Nephrolithiasis	11.4	3.1	8.3
PN	38	Nephrolithiasis	11.0	25.3	24.0
LI	59	Nephrolithiasis	3.2	7.0	11.5
NS	63	Nephrolithiasis	5.8	15.8	18.5
KK	50	Gastric ulcer	7.7	7.4	16.4
SH	40	Gastric ulcer	2.0	2.0	10.8
LH	45	Gastric ulcer	7.5	6.2	11.3
SN	30	Gastric adenoma	2.5	2.7	6.7
HA	41	Duodenal ulcer	8.5	7.0	8.0
MW	48	Diverticulosis coli	2.0	2.0	37.5
HB	45	Myoma uteri	3.5	6.7	17.5
SS	74	Prostatic hypertrophy	7.5	9.7	16.4
NL	76	Prostatic hypertrophy	10.0	22.0	17.1
RF	50	Prostatic hypertrophy	6.1	12.0	15.0
NJ	66	Prostatic carcinoma	5.0	11.0	14.0
LR	74	Hypernephroma	6.0	10.0	11.7
UG	63	Carcinoma of bladder	2.0	18.7	42.9
ST	72	Carcinoma of bladder	12.0	13.9	19.0
ET	55	Carcinoma of penis	7.8	5.0	4.6
AF	38	Carcinoma of breast	4.8	8.4	17.4
BI	54	Carcinoma of breast	10.4	17.5	20.7
Pd	49	Carcinoma of breast	7.0	3.5	10.4
Le	48	Fibroadenoma of breast	2.0	5.3	3.2
		Avg.	*5.53±0.52*	*8.55±1.02*	*14.51±1.46*

the increase in plasma steroids and the magnitude of the operation.

Seven patients had a higher plasma level of steroids on one of the preoperative days then on the day of operation. During the postoperative course, these patients did not differ from the others in the study.

SUMMARY

The adrenocortical activity of 33 surgical patients was investigated in the preoperative period, and evaluated through the determination of 17-hydroxycorticosteroid levels in the peripheral blood.

Twenty-six of 33 patients showed an increase in the concentration of plasma steroids in the preoperative period.

It is suggested that the preoperative elevation of the plasma steroid level is due to psychic tension.

REFERENCES

1. Franksson, C.; Gemzell, C. A., and von Euler, U. S.: Cortical and medullary adrenal activity in surgical and allied conditions, *J. Clin. Endocrinol. & Metab.* **14**: 608, 1954.
2. Roche, M.; Thorn, G. W., and Hills, A. G.: The levels of circulating eosinophils and their response to ACTH in surgery, *New England J. Med.* **242**: 307, 1950.
3. Nelson, D. H., and Samuels, L. T.: A method for the determination of 17-hydroxycorticosteroids in blood: 17-hydroxycorticosterone in the peripheral circulation, *J. Clin. Endocrinol. & Metab.* **12**: 519, 1952.
4. Allen, W. M.: A simple method for analyzing complicated absorption curves, of use in the colorimetric determination of urinary steroids, *J. Clin. Endocrinol.* **10**: 71, 1950.
5. Gemzell, C. A., and Franksson, C.: The effect of ACTH on the blood level of 17-hydroxycorticosteroids and the circulating eosinophils in man, *Acta endocrinol.* **14**: 205, 1953.
6. Tyler, F. H.; Migeon, C.; Florentin, A. A., and Samuels, L. T.: The diurnal variation of 17-hydroxycorticosteroid levels in plasma; (abstract) *J. Clin. Endocrinol. & Metab.* **14**: 774, 1954.
7. Doe, R. P.; Flink, Gay, M., and Flint, B. S.: Correlation of diurnal variations in eosinophils and 17-hydroxycorticosteroids in plasma and urine, (abstract) *J. Clin. Endocrinol. & Metab.* **14**: 774, 1954.

CLINICAL APPLICATION OF THE SIMPLIFIED SILBER-PORTER METHOD FOR DETERMINING PLASMA 17-HYDROXYCORTICOSTEROIDS*

ELEANOR Z. WALLACE, M.D.,† NICHOLAS P. CHRISTY, M.D.‡ AND JOSEPH W. JAILER, M.D.

Departments of Medicine and Obstetrics and Gynecology, College of Physicians and Surgeons, Columbia University and the Presbyterian Hospital in New York City

THE need for practical chemical technics for quantitative estimation of plasma 17-hydroxycorticosteroids has long been recognized, but no clinically feasible methods have been available until the methods of Nelson and Samuels and their co-workers were developed (1, 2). In the relatively short period since their introduction, it has become increasingly apparent that such technics are useful adjuncts to the study of normal and disordered adrenocortical function (3–14). The advantages of procedures applicable to plasma extracts are obvious. They afford a means of assessing adrenocortical function at a given moment and eliminate the inaccuracies of 24-hour urine collections. Evidence has also been obtained suggesting that in certain clinical situations (to be described) plasma values for 17-hydroxycorticosteroid levels are a more accurate reflection of adrenal function than are the urinary values.

It has been demonstrated that 17-hydroxycorticosterone is the chief circulating secretory product of the human adrenal cortex and that this steroid accounts for most of the rise in 17-OH-corticosteroids which follows the administration of ACTH (15, 16). Estimation of 17-hydroxycorticosterone and its congeners in plasma should thus afford valid information about adrenocortical function.

Several methods for determination of free plasma 17-hydroxycorticosteroids have been developed (Table 1). The method most widely used to date has been that of Nelson and Samuels (2), a procedure requiring adsorption of the plasma extract on a Florisil column with subsequent elution. All but two of the technics employ the phenylhydrazine-sulfuric acid

Received for publication December 19, 1954.

* Aided by Grants from the United States Public Health Service, National Institutes of Health, Institute of Arthritis and Metabolic Diseases (A-195); the Upjohn Company; and Merck, Inc.

† Damon Runyon Research Fellow.

‡ Trainee, National Institute of Arthritis and Metabolic Diseases.

reagent of Porter and Silber (21) for identification and quantitation of 17,21-dihydroxy-20-ketosteroids, and are therefore subject to the same limitations of specificity as are inherent in the Porter-Silber reaction itself (19, 21). Methods thus differ from one another in the modes of extraction and purification of plasma. Divergent results reported by various authors are presumably ascribable to variations in the technics of plasma extraction.

Although all the available technics measure free plasma 17-hydroxycorticosteroids, Bongiovanni (22) and Klein *et al.* (23) have claimed the presence of significant amounts of conjugated steroids in plasma, as well.

Silber and Porter (19) have recently described a relatively simple procedure for the measurement of free plasma 17,21-dihydroxy-20-ketosteroids which obviates the use of chromatography, and which can be accomplished in one working day. The following report presents the results of application of this method to the study of normal subjects, hospital patients with non-endocrine diseases and patients with overtly disordered endocrine function.

TABLE 1. NORMAL VALUES OF FREE PLASMA 17-OH-CORTICOSTEROIDS AS REPORTED BY VARIOUS AUTHORS

Author	No. of patients	Plasma 17-OH-CS	
		Range (μg./100 ml.)	Average (μg./100 ml.)
A. *Methods utilizing the Porter-Silber reaction*			
Bayliss & Steinbeck (12)	91*	3–16	9.5±3.3
Bliss *et al.* (3)	120	<2–34	13 ±6
Bondy & Altrock (4)	30	—	7.8±3.3
Ely *et al.* (5)	26†	0–26.8	10 ±1.67
Kassenaar *et al.* (17)	—	—	6.3±1.2
Klein *et al.* (18)	41‡	—	13 ±1.21
Silber & Porter (19)	16	6–25	13.3
Weichselbaum *et al.* (7)	—	8–16	—
Present study	50	4–32	16 ±6.9
B. *Methods not utilizing the Porter-Silber reaction*			
Morris & Williams (6) (polarography)	7	6.5–10.5	—
Sweat *et al.* (20) (fluorescence)	6	7.8–12.8	11

* Includes convalescent patients.

† Children.

‡ Children and adults.

METHODS AND MATERIALS

Technic

A slight modification of the Silber-Porter technic was used. Details of equipment, precision and specificity, and estimates of amounts of steroid lost in handling the extracts have been reported by Silber and Porter (19). The technic employed in the studies here reported is as follows:

1. Five micrograms of 17-hydroxycorticosterone (the free alcohol) in 0.5 ml. of distilled water are added to 10 ml. of heparinized plasma in a 70-ml. centrifuge tube equipped with a plastic cap. The plasma is extracted with 50 ml. of redistilled chloroform by shaking. The tube is centrifuged and the supernatant plasma discarded.

2. The chloroform extract is washed twice—with 4 ml. and 2 ml. of 0.1 N NaOH, and the alkaline washes discarded.

3. The washed chloroform extract is divided into two 20-ml. aliquots. To one of these is added 1 ml. of the Porter-Silber phenylhydrazine-sulfuric acid-ethanol reagent, and to the other, 1 ml. of the blank reagent (sulfuric acid and ethanol without phenylhydrazine).

4. After shaking and centrifugation, the chloroform is discarded. Color development is brought about, either by incubation in a water bath for thirty minutes at 60° C. or by allowing the tubes to stand overnight at room temperature.

5. Standards containing 5 to 20 micrograms of 17-hydroxycorticosterone in 10 ml. of chloroform-washed plasma are carried through the procedure.

6. Samples are read in a Beckman quartz spectrophotometer at 410 mμ in microcuvettes, as described (19). Unknowns are read against the Porter-Silber reagent, and sample blanks against the blank reagent.

Calculation.

$$K = \frac{\text{concentration of standard}}{\text{O.D. standard} - \text{O.D. standard blank}}$$

$$\text{Unknown (in micrograms per 100 ml. plasma)} = [(\text{O.D. unknown} - \text{O.D. unknown blank}) \times K - 5 \text{ gamma}] \times 10.$$

Reproducibility of the method. In general, duplicate samples run simultaneously agreed within an average experimental error of 12 per cent (Table 2). All plasma samples were refrigerated within one hour after blood was drawn. Because of the possibility of changes with time, all determinations were made within seventy-two hours.

A sample experiment is shown in Table 3, to demonstrate the order of magnitude of optical density readings of samples as compared to blanks as a guide to other investigators working with the method.

Clinical material

1. Fifty normal subjects of both sexes were selected from the house and technical staffs of the Columbia-Presbyterian Medical Center. These individuals were in good health and were undergoing the activity of a usual working day.

2. Thirty-one hospital patients, not acutely ill, with miscellaneous nonendocrine diseases were chosen from the ward services of the Presbyterian Hospital. Nineteen additional patients with renal disease were studied.

TABLE 2. COMPARISON OF SIMULTANEOUS DETERMINATIONS OF DUPLICATE SAMPLES

Pair No.	Plasma 17-OH-CS (μg./100 ml.)	
1	5	6
2	11	13
3	10	12
4	20	23
5	20	21
6	21	23
7	38	39
8	41	44

Range of error: 3–20%
Average error: 12.3%

3. Sixteen hospital and clinic patients with endocrine disorders (not adrenal) were investigated.

4. There were 43 patients with adrenocortical disease, including Cushing's syndrome, primary and secondary adrenal insufficiency, and congenital adrenal hyperplasia.

RESULTS

A. *Normal subjects*

Plasma 17-hydroxycorticosteroids were estimated on 66 occasions in 50 normal subjects—28 males and 22 females. The range of values was found

TABLE 3. SAMPLE EXPERIMENT

Sample	Optical density		Corrected optical density	K	Plasma 17-OH-CS (μg./100 ml.)
	of sample	of blank			
10 gamma standard	.206	.010	.196	51.0*	
10 gamma standard	.204	.006	.198	50.5*	
#1	.180	.005	.175		39
#2	.146	.007	.139		21
#3	.148	.012	.136		19
#4	.133	.012	.121		12
#5	.117	.008	.109		5
#6	.124	.003	.121		12
#7	.111	.013	.098		0
#8	.111	.017	.094		0
#9	.149	.018	.131		17
#10	.153	.013	.140		21

* Average K value: 50.8.

to be 4 to 32 micrograms per 100 ml. of plasma, with an average of 16 ± 6.9 micrograms (Fig. 1). Bliss *et al.* (3), who reported the largest group of normal subjects studied to date, found an overall range from less than 2 to 34 micrograms per 100 ml., with an average of 13 ± 6 micrograms. In contrast to the results of that study, a value of less than 4 micrograms per 100 ml. in a normal subject has not been observed in this series, and only 3 of the 66 values were above 28 micrograms. Ranges and average values reported from several laboratories are summarized in Table 1.

In a single normal subject, levels on different days were 20, 29, 7, 26, 32, 19, 19 and 8 micrograms per 100 ml. This finding agrees with the un-

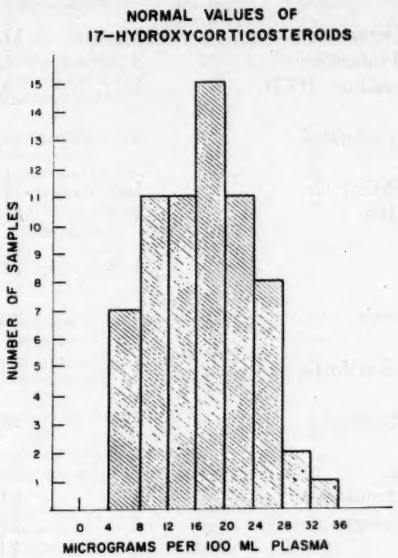

FIGURE 1

explained wide variability in day-to-day levels noted by Bliss *et al.* (3). Because of the reported diurnal variation of 17-hydroxycorticosteroid levels (3, 24), most specimens were obtained in the morning, some in the fasting and some in the post-absorptive state.

B. *Hospital patients with non-endocrine diseases*

In 31 patients not acutely ill and having a variety of disorders, none involving the endocrine glands in any clinically detectable way, the range of values was 4 to 34 micrograms per 100 ml., a spread which is comparable to that in our normal group (Table 4). Perkoff *et al.* (8) reported 17-hydroxycorticosteroid levels in a heterogeneous group of non-endocrine diseases and found a similar range of from 1 to 29 micrograms per 100 ml.

TABLE 4. VALUES OF PLASMA 17-OH-CS IN PATIENTS WITH NON-ENDOCRINE DISEASES

Disease	No. of patients	17-OH-CS (μg./100 ml.)
Carcinoma, ? site	1	12
Carcinoma breast	1	17
Carcinoma uterus	1	9
Laennec's cirrhosis	2	8, 12
Infectious hepatitis	3	18, 10, 19, 17
Rheumatic heart disease	2	17, 14
Myocardial infarction	3	4, 15, 34
Congestive failure, HVD	1	9, 15
Pneumonia, subsided	3	15, 23, 31
TB pericarditis	1	7
TB meningitis	1	19
Obesity	1	31
Psychoneurosis	2	23, 28
Rheumatoid arthritis	1	15
Muscular dystrophy	1	23
GI bleeding	1	21
Lupus erythematosus	1	24
Fever, ? etiology	1	28
Fibroids	2	14, 20
Metrorrhagia	1	24
Cystocele	1	13
Hodgkin's disease, uremia	1	41*

* Determination made shortly before death.

C. *Hospital patients with renal disease*

It has been known for many years that patients with various types of chronic illness exhibit low urinary 17-ketosteroid and corticoid values. Such low levels have been noted in this clinic in patients with renal insufficiency. Since in such cases the question of adrenocortical hypofunction may be raised because of the urinary steroid findings, it seemed important to learn whether plasma corticoid levels in this group were similarly low

when compared with normal subjects. Consequently, studies were made of 19 patients with several types of kidney disease (Table 5). Most patients showed some degree of renal insufficiency as measured by nitrogen retention. The range of plasma 17-hydroxycorticosteroid values in 16 patients was from 5 to 26 micrograms per 100 ml., and 3 additional patients demonstrated elevated values of 34, 39 and 41 micrograms per 100 ml. None of

TABLE 5. COMPARISON OF PLASMA AND URINARY STEROID STUDIES IN PATIENTS WITH RENAL DISEASE

Patient		Diagnosis	Blood urea nitrogen* (mg./100 ml.)	Plasma 17-OH-CS (μg./100 ml.)	Urinary corticoids** (mg./24 hrs.)	Urinary 17-KS (mg./24 hrs.)
Name	Age (yrs.)					
Y.B.	40	Acute glomerulonephritis	16	5	3.4	5.86
M.G.	32	Acute glomerulonephritis	19	26	0.79	1.28
J.J.	35	Acute glomerulonephritis	25	9	3.88	9.7
J.B.	29	Chronic glomerulonephritis, acute exacerbation	20	26	2.9	11.4
M.D.	27	Chronic glomerulonephritis	73	25	0.75	4.33
A.M.	22	Chronic glomerulonephritis	167	12	2.48	1.07
T.H.	46	Chronic glomerulonephritis	51	21	4.85	18.4
E.G.	54	Nephrotic phase, chronic nephritis	11	15	6.67	11.6
M.S.	33	Nephrotic phase, chronic nephritis	8	21	7.65	7.46
M.P.	34	Diabetic nephropathy	28	39	8.9	6.25
H.H.	61	Diabetic nephropathy	—	18	9.7	2.7
J.K.	72	Diabetic nephropathy	64	23	4.16	11.9
A.T.	56	Diabetic nephropathy	28	34	2.2	5.74
C.D.	37	Hypertensive cardiovascular disease, accelerated phase	30	20	3.13	4.45
A.C.	52	Hypertensive cardiovascular disease, accelerated phase	136	23	1.98	2.02
P.G.	54	Nephrocalcinosis	113	17	2.22	2.58
W.D.	53	Periarteritis nodosa	80	26	4.88	5.68
E.C.	33	Amyloid disease	149	41†	.60	1.25
L.T.	64	Polycystic kidneys	31	14	4.6	11.8

* BUN measured in each instance within six days of determination of plasma 17-OH-CS.
** Porter-Silber chromogen after glucuronidase hydrolysis (normal 3–9 mg./24 hrs.).
† Plasma 17-OH-CS determination made within 24 hours of death.

these 3 had clinical manifestations of hyperadrenalism. In some cases, normal levels of plasma corticoids were in notable contrast to low values of urinary 17-ketosteroids and corticoids (Table 5). The explanation of the low urinary values is not as yet established.

D. *Pregnancy*

In 9 patients in the third trimester of apparently normal pregnancies, the range of values was from 22 to 52 micrograms per 100 ml. Gemzell found a mean 17-hydroxycorticosteroid level of 36 micrograms per 100 ml.

at the time of delivery (25). Morris and Williams also reported high values in the pregnant state (6).

E. *Primary adrenal insufficiency*

In 18 patients with Addison's disease, 27 plasma determinations were performed. In nearly all cases, the diagnosis had already been well established on the basis of clinical and other laboratory criteria, some of the patients having been followed in this clinic for periods of from five to twenty years. In this group, the range of values was from 0 to 21 micrograms per 100 ml. (Table 6).

In 10 patients, at least one value of less than 4 micrograms per 100 ml. was obtained. Two patients who had normal amounts of plasma corticoids were given a standard intravenous ACTH test. In neither instance was there any increase in plasma corticoid values. These results will be presented in detail in a subsequent publication (26).

Perkoff *et al.* (8) report a mean value of zero in 7 patients with Addison's disease. In 9 determinations in 3 patients with "partial adrenal insufficiency" the mean was 11 micrograms per 100 ml.

TABLE 6. PLASMA 17-OH-CORTICOSTEROIDS IN ADDISON'S DISEASE

Patient	Medication	17-OH-CS (μg./100 ml.)
J.R.	NaCl	10
F.P.	NaCl	3
Z.McE.	None	6, 13
R.F.	None	21, 16
L.B.	DOC	4, 0
J.C.	DOC	12, 3
A.G.	DOC	9, 3
M.M.	DOC	1
F.K.	DOC	9, 0, 1
R.G.	DOC	3, 1
R.S.	DOC	5
M.Me.	DOC	0
B.N.	DOC	3
W.R.	DOC	0
E.S.	DOC	8
W.S.	DOC	9
C.H.	DOC; 5 mg. cortisone daily, p.o.*	0, 5
F.S.	DOC; 25 mg. cortisone twice daily, p.o.**	4

* Determination made 24 hours after last dose of cortisone.

** Determination made 11½ hours after last dose of cortisone.

F. *Hypopituitarism with secondary adrenal insufficiency*

In 8 patients with classic hypopituitarism, 17-hydroxycorticosteroid levels were determined 11 times. Values ranged from 0 to 12 micrograms per 100 ml. Perkoff and his colleagues (8) reported a range of 0 to 18 micrograms per 100 ml. in patients with hypopituitarism. Bondy and Altrock also found low values (4).

In 3 patients with pituitary tumors, who had no clinical evidence of secondary adrenal insufficiency, plasma levels were normal (11, 13 and 24 micrograms per 100 ml.).

G. *Cushing's syndrome*

Plasma 17-hydroxycorticosteroid determinations were made on 19 occasions in 9 patients with Cushing's syndrome. In each case, the clinical and laboratory diagnosis was borne out by operative or necropsy findings. The range of values was 29 to 107 micrograms per 100 ml. of plasma. Only 2 of the 19 determinations fell within the high normal range (29 and 31 micrograms per 100 ml.), and extraordinarily high values (92 to 107 micrograms per 100 ml.) were found in but 1 patient (H.K., Table 7). Thus most of the values fell within the range of 34 to 57 micrograms per 100 ml. Levels of this order of magnitude have also been reported by Perkoff *et al.* in cases of hyperadrenalism (8). In the present series, the range for the 6 patients with bilateral adrenal hyperplasia was 34 to 107 micrograms per 100 ml.; for the 1 patient with a benign unilateral adenoma, 29 to 39 micrograms; and for the 2 with adrenal carcinoma, 31 to 57 micrograms. Excluding the very high values found in Patient H.K., it is apparent that the ranges of values for the three etiologic categories were not notably different.

Examination of Table 7 shows that in Patients P.T. and F.C. (having adrenal adenoma and hyperplasia, respectively, and both demonstrating the typical clinical features of Cushing's syndrome) 4 of 5 plasma 17-hydroxycorticosteroid values were elevated before therapy, whereas repeated urinary corticoid values, being normal, failed to reflect the clinical status.

The effect of treatment, *i.e.*, adrenalectomy, on plasma 17-hydroxycorticoid levels in patients with hyperadrenalism was of great interest. It will be seen in Table 7 that before treatment all patients had one or more values for plasma corticoid concentration which were distinctly above the normal range. In all patients, regardless of the type of adrenal lesion, when plasma 17-hydroxycorticosteroid levels were determined within a month after unilateral adrenalectomy, normal values were found. (In the instances in which there was supportive therapy with cortisone, this medication was discontinued at least one week before plasma corticoids

were determined, to avoid the effects of inhibition of the pituitary-adrenal axis on the circulating 17-hydroxycorticosteroids.) In only 2 of these patients (P.T. and C.P.) was there clinical evidence of remission immediately following unilateral adrenalectomy. Patient P.T. had a unilateral adenoma and has maintained his remission to date (five months postoperatively). However, after four months Patient C.P., with bilateral adrenal hyper-

TABLE 7. PLASMA AND URINARY STEROID STUDIES IN CUSHING'S SYNDROME

Patient	Urinary corticoids* (mg./24 hrs.)	Plasma 17-OH-CS preoperative (μg./100 ml.)	Plasma 17-OH-CS after unilat. adr'ectomy		Plasma 17-OH-CS after bilat. adr'ectomy	
			Days postop.	(μg./100 ml.)	Days postop.	(μg./100 ml.)
		A. *Adrenal hyperplasia*				
C.P.	15.5	37	14	18	22	0**
			150	29		
K.R.	7.6, 15.5	38–44	18	17	24	12
			35	34	48	12
F.C.	3.5, 3.4, 4.6 3.0†, 3.9, 6.0	37†–44	45	10	—	—
H.K.	47.8	92–107				
S.B.	19.5	—	360	41–46	15	14
S.Bl.	19.2	34–38	7	24	—	—
		B. *Adrenal adenoma*				
P.T.	5.9†, 8.4	29†–39	12	5		
		C. *Adrenal carcinoma*				
M.S.	19.3	—	—	45–57		
I.S.	18.6, 9.4	31–39	35	26	—	—
			65	22		

* Porter-Silber chromogen after glucuronidase hydrolysis (normal 3–9 mg./24 hrs.)
** Specimen obtained at a time when patient exhibited clinical adrenal insufficiency.
† Urine and plasma values measured on the same day.

plasia had clinical evidence of recurrence, although the plasma corticoid level on the one hundred and fiftieth postoperative day was still within the normal range (29 micrograms per 100 ml.). On the other hand, Patient K.R., also with bilateral adrenal hyperplasia, had no evidence of clinical improvement following unilateral adrenalectomy, although her plasma corticoid level was 18 micrograms per 100 ml. on the fourteenth postoperative day. On the thirty-fifth day after operation, the plasma corticoid value rose to 34 micrograms per 100 ml.

In the patients studied after periods greater than one month following surgery, there was better correlation of the plasma 17-hydroxycorticosteroids with the clinical state. Patient F.C., with bilateral hyperplasia, had a value of 10 micrograms per 100 ml. forty-five days after unilateral adrenalectomy, at a time when the disease was in remission clinically. In Patient M.S., with metastatic carcinoma of the adrenal and progression of the signs and symptoms of Cushing's syndrome, plasma 17-hydroxycorticosteroid concentration ranged between 45 and 57 micrograms per 100 ml. In Patient I.S., whose unilateral adrenal carcinoma was surgically removed, values thirty-five and sixty-five days postoperatively were 26 and 22 micrograms per 100 ml. respectively, associated with evidence of moderate clinical improvement. Patient S.B., seen for the first time one year after unilateral adrenalectomy for hyperplasia, had full-blown Cushing's syndrome with plasma corticoid values of 41 to 46 micrograms per 100 ml.

Three patients with adrenal hyperplasia (C.P., K.R. and S.B.; Table 7) were further studied within fifteen to forty-eight days after the second adrenalectomy. Values ranged from 0 to 14 micrograms per 100 ml., and in 2 patients (C.P. and S.B.) were associated with evidences of clinical adrenal insufficiency. In the third patient (K.R.) studied after forty-eight days, there was moderate clinical improvement. Plasma 17-hydroxycorticosteroid values at that time were 12 micrograms per 100 ml.

H. *Congenital adrenal hyperplasia*

Plasma corticoid determinations were made in 9 patients with congenital adrenal hyperplasia and virilism. In all cases, cortisone therapy was withdrawn at least three weeks prior to the time the plasma corticoids were measured. Values ranged from 0 to 20 micrograms per 100 ml. These figures are compared to those reported from other laboratories and summarized in Table 8, which shows the generally low values found here and elsewhere.

TABLE 8. PLASMA 17-OH-CS IN PATIENTS WITH CONGENITAL ADRENAL HYPERPLASIA

Author	No. of patients	Range of 17-OH-CS (μg./100 ml.)
Present study	9	0–20
Bayliss *et al.* (27)	1	3
Bongiovanni *et al.* (13)	2	3–8
	7	less than 1
Ely *et al.* (28)	5	0–8.3
Kelley *et al.* (29)	2	1.6–4.7
Perkoff *et al.* (8)	4	5–14

The subnormal response of these patients to ACTH will be reported in detail in another communication.

I. *Miscellaneous endocrine disorders*

Studies were made in 16 patients with pancreatic, ovarian and thyroid disorders. Exept for 2 patients with diabetic acidosis who had elevated values, the levels ranged from 13 to 30 micrograms per 100 ml. This represents no apparent deviation from the range of our normal group—a finding which agrees with those of Perkoff *et al.* (8) and Bayliss and Steinbeck (12). In Table 9, plasma 17-hydroxycorticosteroid levels in these patients are compared with those found in adrenocortical dysfunction.

TABLE 9. PLASMA 17-OH-CS VALUES IN PATIENTS WITH MISCELLANEOUS ENDOCRINE DISEASES

Disease	No. of patients	No. of determinations	Range of 17-OH-CS (μg./100 ml.)
Addison's disease	18	27	0–21
Hypopituitarism	8	11	0–12
Cushing's syndrome	9	19	29–107
Congenital adrenal hyperplasia	9	9	0–20
Pregnancy, 3rd trimester	9	8	22–52
Diabetes mellitus	7	7	14–30
Diabetic acidosis	2	2	35–36
Turner's syndrome	1	1	16
Hirsutism (females)	4	4	13–21
Thyrotoxicosis	2	2	14–15
Thyroid crisis	1	1	36*

* Determination made shortly before death.

DISCUSSION

The results obtained by the simplified method presented here conform those of other authors (3–5, 8–14, 18, 19) and establish the clinical usefulness of the new Silber-Porter technic (19) for estimating plasma 17,21-dihydroxy-20-ketosteroids.

The value of any new laboratory procedure depends upon 1) its accuracy, 2) the ease with which it can be adapted to conditions in a general hospital laboratory, and 3) its usefulness in distinguishing among various clinical states. The Silber-Porter technic appears to satisfy all three criteria. In this laboratory, the method can be used as a routine diagnostic procedure, and samples from as many as 15 patients can be processed completely in one working day. The elimination of time-consuming steps such as evap-

oration of chloroform extracts to dryness and column chromatography makes possible routine clinical application of the technic.

The method is useful in the clinical diagnosis of disordered adrenal function. In general, plasma 17-hydroxycorticosteroid values of normal subjects, of patients with non-endocrine diseases, and of patients with endocrine disorders not involving the adrenal cortex fall into the normal range. This is in contrast to values of urinary corticoid excretion which may be in the addisonian range in several chronic illnesses. Plasma 17-hydroxycorticosteroid values in patients with adrenocortical dysfunction usually fall outside the normal range.

For example, in untreated Cushing's syndrome, plasma levels of 17-hydroxycorticosteroids were particularly helpful in confirming the diagnosis, and in 2 instances reflected adrenal hyperfunction more accurately than did urinary steroid excretion studies. In contrast to the findings of Perkoff and his co-workers (8), corticosteroid values in the various etiologic categories of hyperadrenalism (hyperplasia, adenoma, carcinoma) did not appear to differ in any characteristic manner.

The constant fall in plasma 17-hydroxycorticosteroid levels which followed unilateral adrenalectomy in patients with Cushing's syndrome due to bilateral adrenal hyperplasia occurred whether or not there was clinical improvement, and could not be used to predict the course of the disease. Following bilateral adrenalectomy in these patients, there was a closer correlation between fall in plasma corticosteroid levels and clinical improvement.

In patients with both primary and secondary adrenocortical hypofunction, the plasma corticosteroid values were generally low. As in the experience of other investigators (8), however, several patients in both categories had values within the normal range. There is no completely satisfactory explanation for this phenomenon in Addison's disease. In both groups the use of an ACTH-plasma corticoid response test appeared to be a more informative index of adrenal insufficiency than any number of single plasma corticoid determinations (26).

There is, in some circumstances, a dissociation between plasma corticoid levels and other clinical or laboratory indices of adrenocortical function. In patients with the adrenogenital syndrome, the occurrence of values ranging between undetectable and normal levels for plasma 17-hydroxycorticosteroids is in accord with the experience of other investigators (8, 13, 27, 28, 29). It remains of interest that patients with adrenal virilism may have no plasma 17-hydroxycorticosteroids, and yet have no evidence of adrenal insufficiency. In the patients with renal disease, normal plasma levels (and in 3 patients, elevated values) were often accompanied by

subnormal urinary corticoid levels of the order usually seen in adrenal insufficiency. In this group, the normal plasma concentrations and the clinical appearance appeared to be more indicative of the state of adrenal function than were the urinary values.

It should be remembered that circumstances other than hyperadrenalism can cause elevations in plasma 17-hydroxycorticosteroids. Renal insufficiency, pregnancy and diabetic acidosis, in this series, and the moribund state (8), anesthesia, surgical trauma (14), and salicylate intoxication (30) have been found to cause increases above normal in plasma corticosteroid levels. The physiologic significance of abnormally high values under such conditions must await further studies of steroid metabolism and excretion, as well as elucidation of the true role of the adrenal cortex in "stress."

SUMMARY

1. A minor modification of the Silber-Porter technic for the measurement of plasma 17,21-dihydroxy-20-ketosteroids is described. Its simplicity renders the technic readily applicable to clinical observations.

2. By this method, the range of values in 50 normal subjects was 4 to 32 micrograms per 100 ml. of plasma, with an average of 16 ± 6.9 micrograms.

3. Normal plasma 17-hydroxycorticosteroid levels were observed in most patients with non-endocrine disease and in patients with disorders of glands other than the adrenal cortex. Elevated values were found in diabetic acidosis, in 3 patients with renal disease and in 2 moribund patients, none of whom exhibited clinical evidence of hyperadrenalism.

4. Patients with renal insufficiency had normal or elevated plasma corticoid levels, in contrast to the abnormally low urinary 17-ketosteroid and corticoid values sometimes observed.

5. The method was especially useful in the diagnosis of hyperadrenalism. In the cases of Cushing's syndrome, the range of values was 29 to 107 micrograms per 100 ml. of plasma, with only 2 of 19 determinations being in the high normal range (29 and 31 micrograms per 100 ml., respectively). In 2 of the cases, elevated plasma corticosteroid levels afforded more accurate measures of adrenocortical status than did the observed normal urinary steroid excretion values. Changes in plasma 17-hydroxycorticosteroid concentration following unilateral and bilateral adrenalectomy in patients with Cushing's syndrome are described.

6. In primary adrenal insufficiency, values ranged from 0 to 21 micrograms per 100 ml. of plasma. In hypoadrenalism secondary to pituitary insufficiency, values were also low, ranging from 0 to 12 micrograms per 100 ml. In these two disorders, a single plasma 17-hydroxycorticosteroid determination often did not furnish sufficient evidence to establish a diagnosis.

REFERENCES

1. Nelson, D. H.; Samuels, L. T.; Willardson, D. G., and Tyler, F. H.: The levels of 17-hydroxycorticosteroids in peripheral blood of human subjects, *J. Clin. Endocrinol. & Metab.* **11**: 1021, 1951.
2. Nelson, D. H., and Samuels, L. T.: A method for the determination of 17-hydroxycorticosteroids in blood: 17-hydroxycorticosterone in the peripheral circulation, *J. Clin. Endocrinol. & Metab.* **12**: 519, 1952.
3. Bliss, E. L.; Sandberg, A. A.; Nelson, D. H., and Eik-Nes, K.: The normal levels of 17-hydroxycorticosteroids in peripheral blood of man, *J. Clin. Invest.* **32**: 818, 1953.
4. Bondy, P. K., and Altrock, J. R.: Estimation of the rate of release of adrenal 17-hydroxycorticosteroids in the human being by the venous catheter technique, with a method for determining plasma 17-hydroxycorticosteroids, *J. Clin. Invest.* **32**: 703, 1953.
5. Ely, R. S.; Kelley, V. C., and Raile, R. B.: Studies of 17-hydroxycorticosteroids in children. I. Peripheral blood levels in health and disease, *J. Pediat.* **42**: 38, 1953.
6. Morris, C. J. O. R., and Williams, D. C.: The polarographic estimation of steroid hormones, *Biochem. J.* **54**: 470, 1953.
7. Weichselbaum, T. E.; Margraf, H. W., and Elman, R.: Quantitative determination of 17-hydroxycorticosteroids and 21-hydroxy-20-ketosteroids in the peripheral blood of humans before and after operative trauma, *Fed. Proc.* **12**: 287, 1953.
8. Perkoff, G.; Sandberg, A. A.; Nelson, D. H., and Tyler, F. H.: Clinical usefulness of determination of circulating 17-hydroxycorticosteroid levels, *Arch. Int. Med.* **93**: 1, 1954.
9. Eik-Nes, K.; Sandberg, A. A.; Nelson, D. H.; Tyler, F. H., and Samuels, L. T.: Changes in plasma levels of 17-hydroxycorticosteroids during the intravenous administration of ACTH. I. A test of adrenocortical capacity in the human, *J. Clin. Invest.* **33**: 1502, 1954.
10. Tyler, F. H.; Schmidt, C. D.; Eik-Nes, K.; Brown, H., and Samuels, L. T.: The role of the liver and the adrenal in producing elevated plasma 17-hydroxycorticosteroid levels in surgery, *J. Clin. Invest.* **33**: 1517, 1954.
11. Brown, H.; Willardson, D. G.; Samuels, L. T., and Tyler, F. H.: 17-Hydroxycorticosteroid metabolism in liver disease, *J. Clin. Invest.* **33**: 1524, 1954.
12. Bayliss, R. I. S., and Steinbeck, A. W.: A modified method for estimating 17-hydroxycorticosteroids in plasma, *in* Memoirs of the Society for Endocrinology, No. 2, London, Dennis Dobson, Ltd., November 1953, p. 31, *et seq.*
13. Bongiovanni, A. M.; Eberlein, W. R., and Cara, J.: Studies on the metabolism of adrenal steroids in the adrenogenital syndrome, *J. Clin. Endocrinol. & Metab.* **14**: 409, 1954.
14. Sandberg, A. A.; Eik-Nes, K.; Samuels, L. T., and Tyler, F. H.: The effects of surgery on the blood levels and metabolism of 17-hydroxycorticosteroids in man, *J. Clin. Invest.* **33**: 1509, 1954.
15. Bush, I. E., and Sandberg, A. A.: Adrenocortical hormones in human plasma, *J. Biol. Chem.* **205**: 783, 1953.
16. Romanoff, E. B.; Hudson, P., and Pincus, G.: Isolation of hydrocortisone and corticosterone from human adrenal vein blood, *J. Clin. Endocrinol. & Metab.* **13**: 1546, 1953.
17. Kassenaar, A.; Molenaar, A.; Nijland, J., and Querido, A.: Estimation of

17-hydroxycorticoids in peripheral human blood, *J. Clin. Endocrinol. & Metab.* **14**: 746, 1954.

18. KLEIN, R.; FORTUNATO, J., and PAPADATOS, C.: Free blood corticoids in the newborn infant, *J. Clin. Invest.* **33**: 35, 1954.
19. SILBER, R. H., and PORTER, C. C.: The determination of 17,21-dihydroxy-20-ketosteroids in urine and plasma, *J. Biol. Chem.* **210**: 923, 1954.
20. SWEAT, M. L.; ABBOTT, W. E.; JEFFRIES, W. M., and BLISS, E. L.: Adrenocortical steroids in human peripheral and adrenal venous blood as determined by fluorescence, *Fed. Proc.* **12**: 141, 1953.
21. PORTER, C. C., and SILBER, R. H.: A quantitative color reaction for cortisone and related 17,21-dihydroxy-20-ketosteroids, *J. Biol. Chem.* **185**: 201, 1950.
22. BONGIOVANNI, A. M.: Detection of corticoid conjugates in human blood, *J. Clin. Endocrinol. & Metab.* **14**: 341, 1954.
23. KLEIN, R.; PAPADATOS, C.; FORTUNATO, J., and BYERS, C.: Conjugated serum corticoids, (abstract) *J. Clin. Endocrinol. & Metab.* **14**: 815, 1954.
24. TYLER, F. H.; MIGEON, C.; FLORENTIN, A. A., and SAMUELS, L. T.: Diurnal variation of 17-hydroxycorticosteroid levels in plasma, (abstract) *J. Clin. Endocrinol. & Metab.* **14**: 774, 1954.
25. GEMZELL, C. A.: Blood levels of 17-hydroxycorticosteroids in normal pregnancy, *J. Clin. Endocrinol. & Metab.* **13**: 898, 1953.
26. CHRISTY, N. P.; WALLACE, E. Z., and JAILER, J. W.: Effect of intravenously administered ACTH on plasma 17,21-dihydroxy-20-ketosteroids in normal individuals and in patients with disorders of the adrenal cortex, *J. Clin. Invest.* **34**: 899, 1955.
27. BAYLISS, R. I. S.; BROADBENT, I. E., and STEINBECK, A. W.: Congenital adrenal hyperplasia. Effects of corticotrophin and tetrahydrocortisone, *Lancet* **1**: 434, 1954.
28. ELY, R. S.; RAILE, R. B.; BRAY, P. F., and KELLEY, V. C.: Studies of 17-hydroxycorticosteroids. IV. Evaluation of a standard ACTH-17-hydroxycorticosteroid response test in children, *Pediatrics* **13**: 403, 1954.
29. KELLEY, V. C.; ELY, R. S., and RAILE, R. B.: Metabolic studies in patients with congenital adrenal hyperplasia. Effects of cortisone therapy, *J. Clin. Endocrinol. & Metab.* **12**: 1140, 1952.
30. DONE, A. K.; ELY, R. S., and KELLEY, V. C.: Studies of 17-hydroxycorticosteroids III. Blood levels in salicylate intoxication, *J. Pediat.* **44**: 153, 1954.

EXTRACTABLE GLUCAGON OF THE HUMAN PANCREAS

A. J. KENNY, M.B., B.S.

Department of Pharmacology and Experimental Therapeutics, The Johns Hopkins University, Baltimore, Maryland

THE presence of a hyperglycemic substance was recognized in some of the earliest preparations of insulin. Murlin suggested the name of glucagon for this substance in 1923 (1). It was, however, the recent work of de Duve (2) and Sutherland (3, 4, 5) which stimulated the current interest. The hyperglycemic-glycogenolytic factor, or glucagon, has been found in pancreatic extracts of all species in which it has been sought (6, 7). In some species, but not all, it has also been found in extracts of gastric mucosa and to a lesser extent in duodenum and ileum (4). It has been postulated that the alpha cells are the main source of this substance in the pancreatic islets (8), though objections have been raised to this view (9). Glucagon has been recently obtained in a highly purified, crystalline form and it appears to have the properties of a protein (10). Its principal, and probably only site of action, is in the liver cell where it increases the amount of the active form of phosphorylase (5). This enzyme appears to be the rate-limiting factor in the glycogenolytic pathway to glucose. It has been suggested that glucagon is a true hormone and since it is a potent agent in carbohydrate and possibly fat metabolism (11, 12), much speculation has arisen concerning its possible rôle in man, particularly in human diabetes. Beyond the finding that glucagon is present in the human pancreas (6), little is known about endogenous glucagon in man. Because of the interesting possibilities of this substance in explaining certain aspects of the diabetic syndrome, it seemed desirable to attempt some direct measurements of glucagon in a series of human pancreases.

METHODS

Experimental

The pancreases used in this study were obtained from routine autopsies at five different hospitals.[1] After the usual gross examination of the organ and removal of tissue for histologic purposes, the bulk of the pancreas was placed in an air-tight container and stored in the frozen state until extraction was possible. The method of extraction was

Received for publication December 19, 1954.

[1] The author wishes to thank Dr. Stanton L. Eversole, Jr., Dr. William Meissner and Dr. Tobias Weinberg for their cooperation in making specimens available for assay.

essentially that of Best, Haist and Ridout (13), with a few modifications. Before complete thawing, the pancreas was dissected free of extraneous tissue, weighed, and placed in a Waring blendor with 5 ml. of acid-alcohol (750 ml. of absolute alcohol, 250 ml. of water, and 15 ml. of conc. HCl) per gram of tissue. The material was homogenized and allowed to stand overnight at 4° C. The homogenate was centrifuged and the sediment re-extracted with 4 ml. of acid-alcohol per gram of pancreas for one hour. After centrifugation it was extracted a third time with 3 ml. of acid alcohol per gram of tissue for half an hour and recentrifuged. The pooled supernatant extract from the three extractions was brought to pH 7.5 with ammonium hydroxide and filtered. The protein fraction containing glucagon was precipitated by the addition of 68 ml. of absolute alcohol and 112 ml. of ether to 40-ml. aliquots of the extract. After standing overnight in a refrigerator the precipitate was separated by centrifugation, dried in vacuo, and the dried powder taken up with 2.5 to 3 ml. of chloride-phosphate buffer per gram of pancreas extracted. The buffer was prepared from one part of 0.1 M phosphate buffer, pH 7.4, and four parts of 0.9 per cent NaCl. After removal of the insoluble portion, the extract was dialyzed against the same buffer for forty-eight to seventy-two hours at 4° C. The extract was made up to a known volume and the final concentration expressed as mg. of pancreas per ml. Satisfactory recovery of known amounts of glucagon by this procedure has been reported (4) and was confirmed during the course of this study.

The assay was based on the method described by Sutherland and de Duve (4). The activity of an extract can be estimated from the increased output of glucose by rabbit liver slices incubated with the extract compared with control slices incubated with the chloride-phosphate buffer alone. Young rabbits, two to three months old, were used. Animals were rejected if more than minimal coccidiosis was evident when the liver was removed. The rabbits were killed by exsanguination following a blow on the head. The liver was removed quickly and placed in ice-cold chloride-phosphate for fifteen minutes before slicing. Slices were cut in a Stadie-Riggs (14) tissue slicer and were kept in a moist cold atmosphere until added to the tubes prepared for incubation. The time taken for slicing and weighing was about forty minutes. The slices were incubated at 37° C. in stoppered 25×150 mm. tubes, shaken in a water bath for one hour. The tubes contained 3 ml. of diluted extract or standard—or, in the case of the controls, chloride-phosphate buffer. Some assays were made using 2 ml. of the medium in the tubes, with incubation for forty-five minutes. Both techniques were satisfactory. Incubation was terminated by immersion of the tubes in ice water, followed by the addition of 0.5 ml. of 0.3 N BaOH and 0.5 ml. of 5 per cent $ZnSO_4$. The protein-free filtrate was analyzed for glucose by the method of Hagedorn and Jensen (15). The response of each slice was expressed as milligrams of glucose per 10 grams of liver per hour of incubation. The values of the control slices were subtracted from those of the paired slices incubated with an extract or standard.

The design of the assay was similar to that used by Audy and Kerly (16). A standard, partially pure, preparation of glucagon[2] (No. E 1433, Lilly) and three pancreatic extracts were assayed concurrently to form a single assay group. A rough estimate of the activity of the extracts was first made in a pilot assay in which three concentrations (10, 40 and 160 mg. per ml.) usually sufficed for each extract. Four concentrations in geometric progression (the second being twice the first, etc.) were then selected for the main assay, so that the responses would be of the same order of size as those of the standard. In practice, the concentrations used ranged from 5 to 40 mg. per ml. in the most active, to

[2] The author wishes to express his thanks to Dr. O. K. Behrens and the Eli Lilly Company for generously supplying the preparation of glucagon used in this study.

40 to 320 mg. per ml. in the least active extracts. The standard was prepared at concentrations of either 1.5 to 12 or 2 to 16 micrograms per milliliter. In the calculation of the relative potency of an extract, 1 microgram of the standard was arbitrarily equated with 10 mg. of pancreas. Thus, if an extract was found to be of the same potency as the standard, it would follow that the pancreas contained glucagon equivalent to 100 micrograms of the standard per gram of pancreas. A stock solution of the extract was prepared, containing 1 mg. per ml. in 0.0033 N HCl. It was kept in sealed tubes at −18° C. Each tube contained a sufficient amount to prepare the dilutions needed for each day's assay. There was no loss of activity during storage over a period of two months.

Eight liver slices 0.5 mm. thick and weighing about 250 mg. were cut and randomly allocated to the standard and the three extracts. Each slice was divided into three pieces weighing between 58 and 82 mg. (moist weight). One of the three was a control slice and the other two were incubated with either the lowest and the highest, or with the two intermediate concentrations of the extract or standard. Thus the twenty-four slices which were cut from each rabbit liver, comprised eight control slices and four slices for each of the three extracts and the standard, *i.e.*, one slice at each concentration. Replicates of the response at each concentration were obtained by repeating this design on subsequent days, so that, in all, from 4 to 6 animals were used in each assay group.

Calculation and analysis of the assay results

If the log. concentration-response lines are rectilinear and parallel, the logarithm of the relative potency can be estimated by the distance between the lines of the standard and an extract, measured on the log. concentration scale. This value, M, the logarithm of the relative potency, may also be obtained in these circumstances by the formula (16):

$$M = \bar{X}_s - \bar{X}_u - \frac{(\bar{Y}_s - \bar{Y}_u)}{b}$$

where $\bar{X}$ is the mean log. concentration and $\bar{Y}$ the mean response, the subscripts s and u refer to the standard and the unknown extract, and b is the slope of the line fitted by the method of least squares. The data for all four lines were pooled to provide a mean value of the slope. In these assays linearity and parallelism were assumed, and the statistical analysis of the data was limited to obtaining an estimate of the error of the assay. Somewhat crude methods of analysis were used, since highly precise statistical methods are inappropriate unless the data are of similar precision. The foregoing formula for M was applied to the results from each repetition of the assay on different rabbit livers. Thus, in any one assay group, several values of M were obtained, each representing a particular extract assayed on a particular liver. With three extracts and four repetitions there were twelve such values. These values were examined in a simple two-dimensional analysis of variance, in which sources of variation were attributed to rabbits and to extracts and the residual mean square was regarded as the error variance (s^2). However, the precision of a value of M is affected by both the error variance, s^2, and the variance attributable to rabbits, s_R^2. Now the theoretical value for the mean square for rabbits in the analysis of variance is $e \cdot s_R^2 + s^2$, where e is the number of extracts in an assay group—always three in this series. Hence s_R^2 can be calculated from the obtained value of the mean square for rabbits:

$$s_R^2 = \frac{(\text{obtained mean square}) - s^2}{e}.$$

The variance of M, s_M^2, in an assay based on data from n rabbits (usually 4), and with one repetition of the assay per rabbit, is given by the formula

$$s_M^2 = \frac{s^2 + s_R^2}{n}.$$

The standard error of M, s_M, may be calculated by extracting the square root. The 95 per cent confidence limits of a value of M were computed as $M \mp 2 \cdot s_M$.

RESULTS

The results of the assays are expressed as the logarithm of the relative potency, M, because in this form the distribution of the results was less skewed than when expressed in the antilogarithm form, R. An R of 1.0 or an M of zero corresponds to a concentration of 100 micrograms of "standard" glucagon per gram of pancreas. It might seem preferable to relate the values obtained to the weight of the whole pancreas, so as to estimate total pancreatic extractable glucagon. Such an estimate, when related to body weight or surface area might be a more valuable physiologic measure than a simple expression of the concentration. Except for a few pancreases, this was not practicable because accurate information concerning the weight of the original pancreas was lacking. The portion delivered for assay varied from, perhaps, three-fifths to nine-tenths of the total. The weight recorded at the time of autopsy, when this was known, frequently included the weight of adherent adipose tissue. For these reasons it seemed better to leave the results in their present form, rather than introduce further errors by attempting this conversion. Since the variation in the values of M was large in relation to any likely variation in pancreatic weights, it seems improbable that the interpretation of the results would have been different had this additional information been available.

Thirty-five pancreases were assayed. The results are summarized in Table 1. The mean value of the log. relative potencies was -0.5398 and the median, -0.3087. The corresponding values of R were 0.288 and 0.491 respectively (equivalent to 288 and 491 micrograms of "standard" glucagon per gram of pancreas). The variation among the pancreases was large —more than could be accounted for by the error of the assay method alone. Figure 1 shows the individual assay results together with the 95 per cent confidence limits of each. The mean of the error variances of M was 0.0634 and this value may be compared with the variance of the whole distribution, 0.7716.

Extraneous variables

The use of human autopsy material from several sources introduced certain variables of a type which could be avoided in a planned animal experiment. Between the time of death and the completion of the assay of the pancreatic extract a period of time elapsed which varied from pancreas to pancreas. The postmortem examination was made after a delay of from two to eighteen hours after death. The course of the examination

involved a further delay before the organ was removed, examined and finally placed in a container for freezing. Then followed a variable period of storage while frozen. Finally, a somewhat variable time elapsed between extraction of the pancreas and the completion of the assay. Three of these variables were measured, but the fourth, the period between the start of the autopsy and the freezing of the pancreas, was unknown. Although

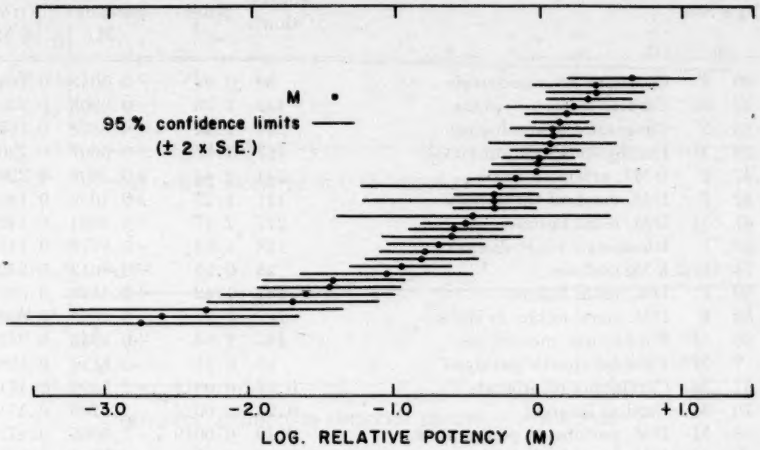

FIG. 1. *Distribution of log. relative potencies of all pancreases, showing 95 per cent confidence limits for each assay.*

there is no reason to suppose that there were any wide fluctuations in this delay, the fact that it was unmeasured precluded any estimate of its influence on the assay results. The influence of the other factors could be assessed and is shown in Figure 2. The correlation coefficients between these variables and the assay results were not in any instance significantly different from zero. Most of the pancreases were obtained from two hospitals. Only 5 pancreases came from the remaining three hospitals. A comparison of the results of the assays of the specimens from the two main sources of supply did not reveal any significant difference between the means.

A review of the histologic sections of 27 of the 35 pancreases showed that 7 had evidence of autolysis. This group had a mean log. relative potency of -0.7644, and the non-autolyzed pancreases a mean of -0.4772. The difference was not significant. There was no indication, therefore, that the lowest assay results were the result of postmortem autolytic changes.

Age and sex

There was a slight tendency for the values of M to fall with increasing age at death (Fig. 3a). The correlation coefficient for the whole group,

TABLE 1. SUMMARY OF MAIN CLINICAL FEATURES AND RESULTS OF ASSAYS OF PANCREATIC GLUCAGON

Patient			Main clinical features	Assay results			
No.	Age	Sex		Concentration*	Relative potency	Log. relative potency (M)	Standard Error of M
1	50	F	Coronary arteriosclerosis	99	0.99	−0.0045	0.1646
2	42	M	Cardiac infarct, syphilis	123	1.23	+0.0903	0.2202
5	15	F	Congenital heart disease	244	2.44	+0.3878	0.1646
6	55	M	Emphysema, cor pulmonale	467	4.67	+0.6697	0.2202
7	47	F	DM†, arteriosclerosis	244	2.44	+0.3869	0.2202
8	42	F	DM, cerebral thrombosis	127	1.27	+0.1036	0.1432
9	47	M	DM, renal failure	217	2.17	+0.3364	0.1432
10	49	F	Rheumatic heart disease	158	1.58	+0.1979	0.1432
11	74	F	DM, acidosis	25	0.25	−0.6013	0.2439
16	50	F	DM, renal failure	142	1.42	+0.1522	0.0994
17	53	F	DM, renal failure,syphilis	127	1.27	+0.1025	0.0994
18	46	M	Pneumonia, meningitis	184	1.84	+0.2643	0.2439
19	7	M	Familial spastic paralysis	15	0.15	−0.8170	0.1980
22	57	M	Carcinoma of prostate	0.18	0.0018	−2.7490	0.4710
23	70	M	Cardiac infarct‡	0.24	0.0024	−2.6209	0.4710
24	58	M	DM, perforated peptic ulcer	0.49	0.0049	−2.3068	0.4710
25	47	M	DM, hepatic cirrhosis	95	0.95	−0.0223	0.1609
27	50	M	Pontine hemorrhage‡	32	0.32	−0.4972	0.1609
28	79	M	Cerebral hemorrhage	51	0.51	−0.2956	0.1609
29	83	M	DM, bronchopneumonia	8.8	0.088	−1.0536	0.2952
30	72	M	DM, cardiac infarct	2	0.02	−1.7017	0.2952
31	37	M	DM (juvenile onset), renal failure	2.5	0.025	−1.6094	0.2952
32	61	M	Bronchopneumonia‡	3.7	0.037	−1.4380	0.2524
33	63	M	Cardiac infarct	3.7	0.037	−1.4295	0.2524
34	67	F	DM, cardiac infarct	114	1.14	+0.0557	0.1296
35	54	F	Arteriosclerotic nephritis‡	102	1.02	+0.0071	0.1296
46	22	F	DM (juvenile onset), renal failure	53	0.53	−0.2749	0.4707
47	81	M	Coronary arteriosclerosis	49	0.49	−0.3087	0.4707
48	67	F	DM acidosis, pyelonephritis	36	0.36	−0.4464	0.4707
49	59	F	DM, carcinoma of lung	70	0.70	−0.1652	0.1676
50	86	M	Cardiac infarct	12	0.12	−0.9399	0.1676
51	56	M	DM, cardiac infarct	21	0.21	−0.6791	0.1676
52	79	F	DM, amputation, pulmonary embolism	16	0.16	−0.7938	0.1342
53	60	M	DM, hemochromatosis	49	0.49	−0.3092	0.1342
54	68	F	DM, bronchopneumonia	26	0.26	−0.5849	0.1342

* Concentration expressed as micrograms of "standard" glucagon per gram of pancreas extracted.

† Diabetes mellitus.

‡ Clinical data insufficient to classify as to diabetes mellitus.

($r = -.28$) did not reach a value significantly different from zero. However, the trend was more marked among the 15 females in the series. This correlation coefficient ($r = -.67$) was significantly different from zero ($P < .01$). The slope of the regression line was the same as that for the whole group —a fall in M of .014 for each year of age. When the results were grouped

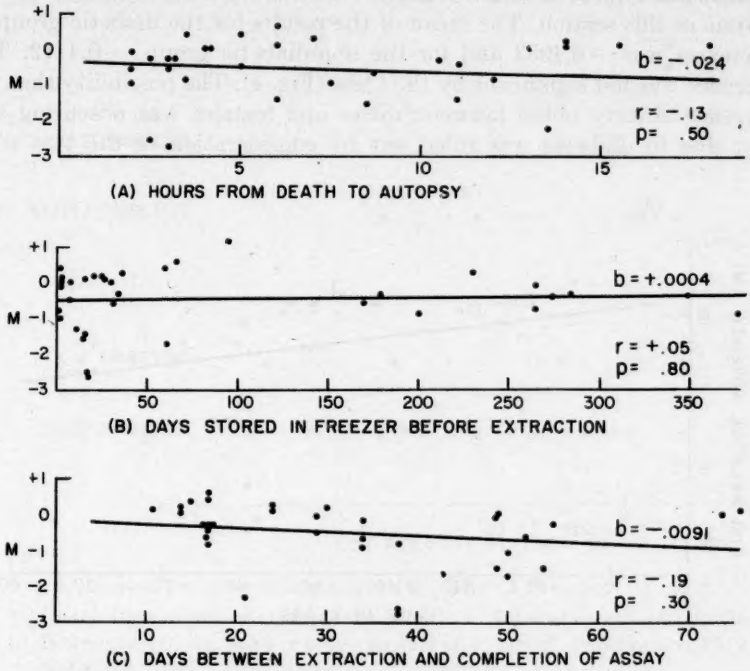

FIG. 2. *Relationship of certain extraneous variables to extractable glucagon.*

a) Correlation between log. relative potency (M) and hours between death and autopsy.

b) Correlation between log. relative potency (M) and days of storage in freezer.

c) Correlation between log. relative potency (M) and days from start of extraction of pancreas to completion of the assay.

r = correlation coefficient; p = probability that r could be zero; b = slope of regression line fitted by method of least squares.

according to sex, a significant difference ($P = .01$) was found (Fig. 3b). The mean for males was -0.8709 and that for females, -0.0985. The distributions of the two groups overlap and it is apparent that there was little variation within the female group, whereas within the male group, which contained several low results, there was a large variance.

Diabetes mellitus

The pancreases assayed in this series represented a selected group so far as diabetes was concerned. Nineteen pancreases from proven diabetics and 12 from patients in whom the diagnosis could be definitely excluded were assayed. There remained 4 patients whose clinical records were equivocal or inadequate for a definite decision and these were excluded from the analysis in this section. The mean of the results for the diabetic group of pancreases was −0.4953 and for the non-diabetic group, −0.4112. The difference was not significant by the *t* test (Fig. 4). The possibility that the difference already noted between males and females was obscuring any effect due to diabetes was ruled out by consideration of the two sexes

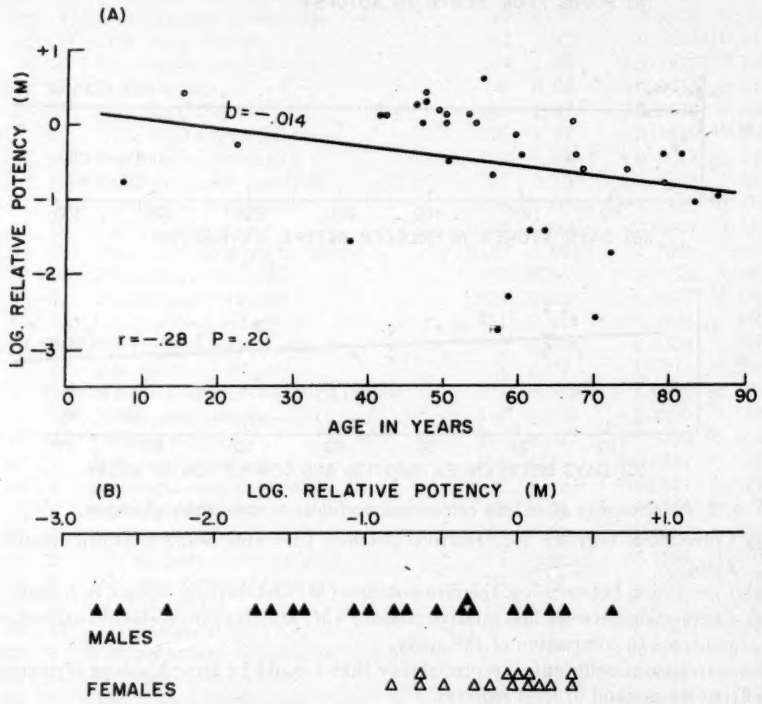

FIG. 3. *Relationship of age and sex to extractable glucagon*

a) Correlation of log. relative potency (M) with age of patient at death. (● Males ○ Females)

b) Distribution of log. relative potencies in males and females.

r = correlation coefficient; *p* = probability that *r* could be zero; *b* = slope of regression line fitted by method of least squares.

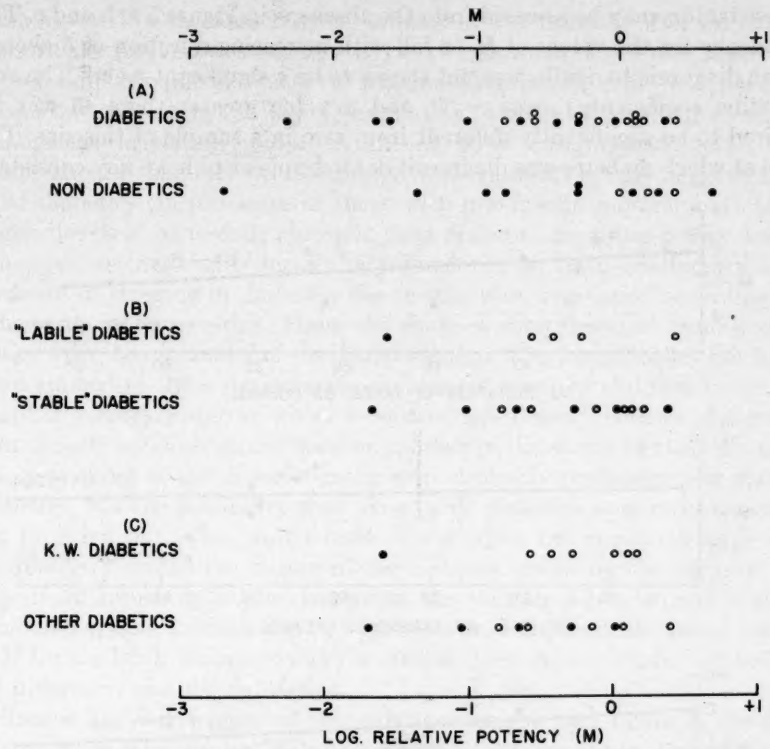

FIG. 4. *Diabetes and extractable glucagon* (● Males ○ Females)

a) Distribution of log. relative potencies (*M*) of 19 diabetics and 12 non-diabetics.

b) Distribution of log. relative potencies (*M*) of 5 "labile" diabetics and 12 "stable" diabetics.

c) Distributions of log. relative potencies (*M*) of 7 patients with pathologic evidence of Kimmelstiel-Wilson lesions and 10 diabetics without such lesions.

separately.[3] The mean for male diabetics was −0.9182 [8] and for male non-diabetics, −0.6128 [9]. For females the values were −0.1878 [11] and +0.1973 [3] respectively, the number of pancreases in each group being shown in brackets. These differences were not significant.

The diabetics in this series showed wide variations in the duration of diabetes, age at onset and daily insulin dosage. The range of values for

[3] In subsequent analyses of the results, this same possibility was considered. In Figures 4 and 5 results for males and females are indicated by different symbols. There is no suggestion that the two sexes exhibit different trends in relation to the other variables considered.

these factors may be assessed from the abscissae in Figure 5 a, b and c. The tendency for the values of M to fall with increasing duration of diabetes, from diagnosis to death, was not shown to be a significant trend. The correlation coefficient, r, was $-.30$, and a value greater than .43 was required to be significantly different from zero in a sample of this size. The age at which diabetes was diagnosed did not appear to have any consistent

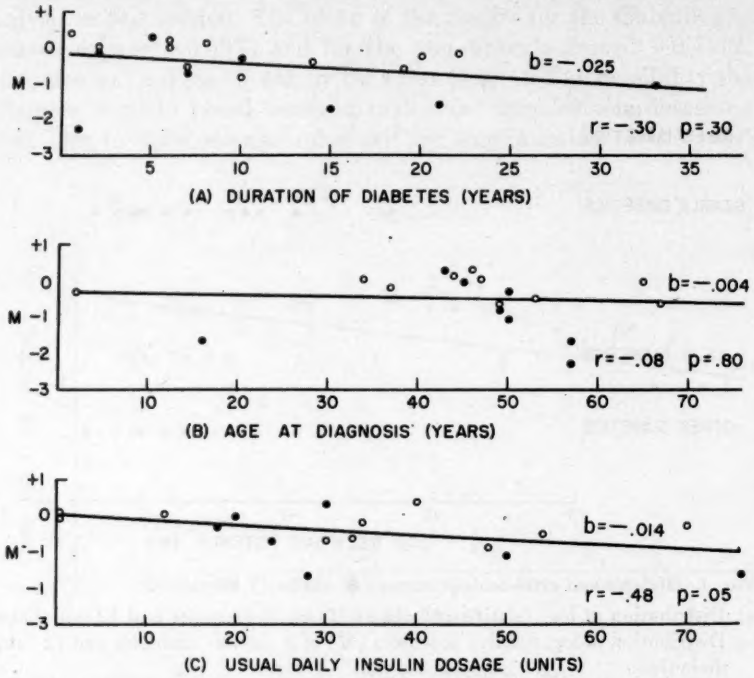

FIG. 5. *Duration, age at diagnosis and insulin dosage; relationship to extractable glucagon* (● Males ○ Females)

a) Correlation of duration of diabetes (years) from diagnosis to death, and log. relative potency (M).

b) Correlation of age at diagnosis (years) and log. relative potency (M).

c) Correlation of usual daily insulin dosage (units) and log. relative potency (M).

r = correlation coefficient; p = probability that r could be zero; b = slope of regression line fitted by method of least squares.

effect on the assay results. An inverse relationship was found between the daily insulin dosage and the values of M; the correlation coefficient ($r = -.48$) reached a significant level ($P = .05$). The insulin dosage recorded was that which the patient usually took in the period prior to the terminal illness. When this preterminal value was not known, the usual daily dose

administered in the final hospital admission was utilized, after excluding abnormally high doses administered in the treatment of acidosis. Since the correlation only just exceeded the minimum significant value, it is possible that small changes in the way of designating the "usual" insulin dose would change the correlation from a "significant" to an "insignificant" value. The result should therefore be interpreted with caution. It may suggest that mild diabetics (in the sense of those with low insulin requirement) have higher levels of pancreatic glucagon than diabetics requiring higher doses. However, as the level of insulin requirement is far from satisfactory as a measure of severity in diabetes, the results were regrouped according to other criteria for severity. Figure 4b shows a comparison of 5 labile diabetics with the remainder of the diabetic group. The term "labile" has here been applied to those patients whose diabetes began in childhood (age 16 years or younger) and/or whose record revealed clear evidence of significant diabetic acidosis at one time or another in the course of their disease. The remainder of the diabetic group were probably predominantly stable diabetics, but the possibility that some labile diabetics were included cannot be ruled out. This would have arisen when the clinical records inadequately revealed the nature of the diabetes, either by the omission of episodes of ketosis or because treatment was continuously adequate to prevent such episodes. When the two groups were compared, the mean value of M for the labile diabetics was -0.5090 and for the remainder -0.3875; the difference was not significant.

Since it has been suggested that glucagon might be a factor in the development of Kimmelstiel-Wilson disease (17), the renal histology of most of the diabetics was reviewed by one pathologist and the sections classified according to the presence of the typical lesions. In 2 patients Kimmelstiel-Wilson lesions were well marked and extensive. In 5 others a probable diagnosis was reached, but the typical picture was partially obscured by other renal pathologic conditions. The mean of the assay results from these 7 patients was -0.4359 and this value was not significantly different from the mean in 10 other patients without such lesions, -0.4884 (Fig. 4c).

Two other clinical states are perhaps worthy of mention. Two patients died in diabetic acidosis and the values for M for these pancreases were -0.6013 and -0.4464. Hemochromatosis was confirmed at autopsy in 1 diabetic. In this case the value of M was -0.3092. None of these values suggests that these conditions are associated with a marked deviation in pancreatic glucagon from the average.

DISCUSSION

A method which attempts to measure the amount of a substance capable of being extracted from a tissue has certain inherent limitations. The ex-

perimental approach is static and reveals nothing of the rates of production and secretion of the substance into the circulation. Since glucagon acts at a site removed from the tissue of its origin, it might be preferable to measure circulating glucagon rather than glucagon stores in the secreting tissue. In the case of insulin these two different approaches, the assay of extractable pancreatic insulin (18) and the assay of plasma insulin (19), led to broadly similar conclusions concerning different types of human diabetes. It might be hoped, but should not be assumed, that the same would hold true for glucagon. However, the evidence that glucagon is a hormone is not yet complete. The present techniques of extraction and assay have failed to detect glucagon in plasma from either peripheral or pancreatic veins, even when concentrated many fold by lyophilization[4] (4, 20). Cross-circulation experiments suggest that glucagon may well be secreted into the pancreatic veins following the stimulus of hypoglycemia or growth hormone (21, 22). A more sensitive assay system might be needed for a successful assay in peripheral blood.

A second limitation is that extractable glucagon in the pancreas may represent only a part of the total present. If so, it would be inferred that the remaining glucagon was, for some unknown reason, inaccessible or irremovable by the extraction procedure; or, alternatively, that part of the pancreatic stores of glucagon existed in an extractable but inactive form. These objections are of a theoretical nature and cannot be answered by the experimental methods used. So far as extractable glucagon is concerned it appeared to be almost completely extracted by the procedure used. Only traces of glucagon activity were present in the tissue residue when further extractions with acid alcohol were made.

The assay technique, too, may have certain limitations concerning both the specificity and precision of the methods used. The assay system is not specific for glucagon. Epinephrine and related sympathomimetic amines exert a glycogenolytic action by the same mechanism as glucagon (5). Reducing substances if present in an extract would give rise to factitious activity. Amylase may exert a glycogenolytic effect, independent of the phosphorylating enzymes. However, preliminary dialysis of the extracts was shown to eliminate both reducing substances and added epinephrine. Amylase—at least that present in plasma—is not extracted, or does not withstand the extraction, with acid alcohol (20). It seems unlikely, therefore, that any of the foregoing substances were present in significant amounts in the extracts. Moreover the general parallelism of the log. con-

[4] Incubation of liver slices with plasma directly, without preliminary extraction, leads to a large output of reducing sugar. This apparent glycogenolytic activity is to be distinguished from that due to glucagon. It is presumably the result of the action of plasma amylase on glycogen derived from the liver slices (20).

centration-response lines of the standard and the extracts suggested that the activity was probably the result of the same substance in each case.

The use of the glycogenolytic effect, rather than the hyperglycemic effect, as the basis of the assay system was the result of some preliminary observations with both methods. The response of an anesthetized cat is a standard method for the assay of the glucagon (10). The cat appears to be a slightly more sensitive preparation than the rabbit liver slice. Moreover, a single observation from a cat has probably greater stability than a single response from a liver slice. Nevertheless, the liver-slice method is sufficiently rapid to enable a satisfactory experimental design to be followed and the error to be calculated. It is unaffected by the presence of insulin in the samples. As sensitivity was not a problem, these considerations outweighed the possible advantages of the cat method.

The error of the assay technique, as estimated from the pooled data of all the assays, indicates some lack of precision. This seems to have been the experience of others who have applied statistical analysis to data derived from this procedure. The standard errors of the log. relative potencies in one series of multiple-dose assays of glucagon in insulin samples (16) showed a wide range in values, more than encompassing the values obtained in this study. The major part of the error variance must be accounted for by the variation between liver slices—in part "biologic," in part the result of undefined variation in the manipulation of the slices. Duplicate analyses of the filtrate for glucose, when made, differed on the average by 1.5 per cent, with a maximum difference of 4 per cent. Any lack of precision in an assay method must represent a further limitation. Small differences between pancreases could be obscured by imprecise measurement of their potencies and it is desirable to make some quantitative estimate of this effect. The error variance of the assay may be considered as entering into the variance of any grouping of the pancreases. For example, if it be assumed that the only variation around the means of two subgroups is that attributable to the error of the assay, it is possible to estimate the minimum difference between the subgroup means which would be statistically significant.[5] In the comparison of the diabetic and non-diabetic subgroups, and assuming this hypothetical situation, a difference greater than 0.1859 would be required to achieve significance at the 5 per cent level. The minimum difference varies slightly with the size and composition of the subgroups under comparison. In the comparison of labile and stable diabetics a difference greater than 0.2680 would be necessary. To express these figures in another way, a difference in means of about 150 to 200 micrograms per

[5] This is approximately given by $2\sqrt{0.0634(1/n_1+1/n_2)}$ for a level of significance with $P=.05$, where 0.0634 is the mean of the error variances of M, and n_1 and n_2 are the numbers of pancreases in the two sub-groups under comparison.

gram of pancreas would be the minimum required for statistical significance. The assumption involved in these estimates represents an unreal situation. In addition to variation due to errors in measurement (here considered to be the only source of variation) there are many other sources of variation which cause differences in the results from different pancreases; and, in so far as these additional sources of variation are not attributable to known factors, they, too, will further tend to obscure differences between subgroups.

The results confirm the presence of glucagon in the human pancreas. The average values appear to be in the same range as those reported for other species. Frequently these values have been expressed as the weight of pancreas (mg. per ml.) inducing a half-maximal response by the liver-slice system. The median value for the human pancreases can be expressed in this form as about 60 mg., and the mean as about 100 mg. For comparison the values reported for other species are: dog, 50 mg.; rabbit 25 to 60 mg.; cat, 25 to 50 mg.; ox, 40 mg.; fetal calf, 10 mg.; hooded rat, 50 mg.; guinea pig, 100 mg.; and the goose fish (islet-tissue), 60 mg. (4, 7).

The idea that glucagon may play a major part in the pathogenesis of diabetes mellitus has received support from several sources. The experimental evidence is, however, indirect and much of it rests on speculation. The different symptomatology in the various types of experimental diabetes has led to the postulate that a pancreatic factor, in addition to insulin, is involved. It is well established that subsequent pancreatectomy in dogs can modify the diabetic state previously induced with alloxan (23), anterior pituitary extract (24), or by partial pancreatectomy (25). Following complete removal of the pancreas the insulin requirement falls to a lower level, insulin sensitivity increases and fatal acidosis can be precipitated if insulin is withheld. In man it has been noted that total pancreatectomy results in a diabetic state with an insulin requirement often in the range of 30 to 40 units per day, a dosage frequently exceeded in spontaneously occurring diabetes. In at least 2 patients (26, 27) diabetes had been present prior to removal of the pancreas but the effect on insulin dosage was less striking than that seen in the dog, the requirement being unchanged in one and increased in the other patient. It must be admitted that the interpretation of these changes is complicated by the profound disturbance in protein and fat digestion and absorption resulting from the absence of the exocrine secretion. Thus the nutritional state following pancreatectomy could of itself produce some of the effects described. Nevertheless, the results have generally been explained as indicating that the pancreas modifies the diabetic state by means of an anti-insulin factor. With the renewed interest in glucagon as a probable hormone of the pancreas there came the suggestion that glucagon was of importance in

modifying the diabetic syndrome. Dana, Eversole and Zubrod (17) suggested that glucagon might be involved in the development of the vascular lesions of Kimmelstiel-Wilson disease and of diabetic retinopathy. Pincus (28) speculated earlier that glucagon could explain the differences between the labile and stable diabetics, since these groups bear some analogy to depancreatized and alloxanized diabetic animals. If the labile patient were deficient in glucagon then his insulin sensitivity, lower insulin requirement and greater tendency to ketosis might be accounted for. Conversely, an excess of glucagon might explain these features in the stable diabetic, who is insensitive to insulin (often requiring large doses), rarely shows ketosis and unlike the labile diabetic, appears to have significant amounts of insulin in both pancreas and plasma. Pincus (28) and, later, Kirtley *et al.* (29, 30) attempted to test this hypothesis by administering glucagon to groups of human diabetics. The results of the two investigations were conflicting. Pincus found that the labile group had a greater hyperglycemic response than the stable group. He argued that the former lacked glucagon and that liver glycogen was inadequately mobilized, but when a small amount of glucagon was injected a greater than normal hyperglycemic response occurred. Kirtley *et al.*, using fasting diabetics, observed a smaller hyperglycemic response in the labile diabetic group and suggested that this might be attributable to lower hepatic glycogen stores. In any case, so many variables might be expected to influence the response in this type of study that it would be hazardous to make any inferences about the activity of endogenously produced glucagon in diabetes. Saka (31) has claimed that a glucagon-like substance occurs in the urine of human diabetics and alloxan diabetic animals but is absent from the urine of non-diabetics. This finding could not be confirmed when urine samples from 6 diabetics showing heavy glycosuria were extracted and assayed by the liver-slice method (20).

It was hoped that the present investigation would throw some light on the relationship of glucagon to human diabetes. The results of the pancreatic assays do not provide any support for the views discussed here. It must be noted, however, that the lack of positive findings does not rule out the possibility that glucagon may play an important rôle in the pathogenesis of diabetes, but at present this will remain a matter of speculation until elucidation is possible by further direct experimentation.

SUMMARY

1. Thirty-five human pancreases, obtained at autopsy, were assayed for extractable glucagon (hyperglycemic glycogenolytic factor).

2. Extracts of the pancreases were compared with a standard, partially pure, preparation of glucagon in a series of multiple-dose assays, using surviving rabbit liver slices as the assay system.

3. The presence of glucagon in the human pancreas was confirmed. There was a large variation among the values of the relative potencies of the pancreatic extracts assayed.

4. Pancreases from females appeared to contain significantly more glucagon than those of males. There was a tendency for extractable-glucagon levels to fall with increasing age, but this correlation was significant only among the females.

5. No significant differences were found between 19 diabetics and 12 non-diabetics, nor between various categories within the diabetic group.

6. Although not excluding such a relationship, the results provide no support for the view that glucagon is a major factor in the pathogenesis of diabetes mellitus.

Acknowledgment

The author wishes to thank Dr. C. G. Zubrod, at whose suggestion this study was begun, for much helpful advice and criticism. The author is also indebted to Mr. Roebert L. Stearman for his advice on statistical methods and to Dr. Stanton L. Eversole, Jr. for reviewing the histologic preparations.

REFERENCES

1. KIMBALL, C. P., and MURLIN, J. R.: Aqueous extracts of pancreas, *J. Biol. Chem.* **58**: 337, 1923.
2. DE DUVE, C.; HERS, H. G., and BOUCKAERT, J. P.: Nouvelles recherches concernant l'action de l'insuline, *Arch. internat. pharmacodyn.* **72**: 45, 1946.
3. SUTHERLAND, E. W., and CORI, C. G.: Influence of insulin preparations on glycogenolysis in liver slices, *J. Biol. Chem.* **172**: 737, 1948.
4. SUTHERLAND, E. W., and DE DUVE, C.: Origin and distribution of the hyperglycemic-glycogenolytic factor of the pancreas, *J. Biol. Chem.* **175**: 663, 1948.
5. SUTHERLAND, E. W., and CORI, C. F.: Effect of hyperglycemic-glycogenolytic factor and epinephrine on liver phosphorylase, *J. Biol. Chem.* **188**: 531, 1951.
6. SUTHERLAND, E. W.: The effect of the hyperglycemic factor of the pancreas and of epinephrine on glycogenolysis, *Recent Progr. Hormone Res.* **5**: 443, 1950.
7. AUDY, G., and KERLY, M.: The content of the glycogenolytic factor in pancreas from different species, *Biochem. J.* **52**: 77, 1952.
8. HEARD, R. D. H.; LOZINSKI, E.; STEWART, L., and STEWART, R. D.: An α cell hormone of the islets of Langerhans, *J. Biol. Chem.* **172**: 857, 1948.
9. VOLK, B. W.; LAZARUS, S. S., and GOLDNER, M. G.: Alpha cells of the pancreas; morphologic and physiologic considerations, *A. M. A. Arch. Int. Med.* **93**: 87, 1954.
10. STAUB, A.; SINN, L., and BEHRENS, O. K.: Purification and crystallization of hyperglycemic-glycogenolytic factor, *Science* **117**: 628, 1953.
11. HAUGAARD, E. S., and STADIE, W. C.: The effect of hyperglycemic factor and epinephrine on fatty acid synthesis, *J. Biol. Chem.* **200**: 753, 1953.
12. KALANT, N.: Metabolic effects of the pancreatic hyperglycemic factor, *Proc. Soc. Exper. Biol. & Med.* **86**: 617, 1954.
13. BEST, C. H.; HAIST, R. E., and RIDOUT, J. H.: Diet and the insulin content of pancreas, *J. Physiol.* **97**: 107, 1939.

14. STADIE, W. C., and RIGGS, B. C.: Microtome for the preparation of tissue slices for metabolic studies of surviving tissues in vitro, *J. Biol. Chem.* **154**: 687, 1944.
15. PETERS, J. P., and VAN SLYKE, D. D.: Quantitative Clinical Chemistry. Volume II. Methods. Baltimore, The Williams and Wilkins Company, 1932, p. 471.
16. AUDY, G., and KERLY, J.: The content of glycogenolytic factor in some insulin samples, *Biochem. J.* **52**: 70, 1952.
17. DANA, G. W.; EVERSOLE, S. L., and ZUBROD, C. G.: Hyperglycemia and its relation to diabetic retinitis and glomerular nodules, *Bull. Johns Hopkins Hosp.* **90**: 98, 1952.
18. WRENSHALL, G. A.; BOGOCH, A., and RITCHIE, R. C.: Extractable insulin of pancreas, *Diabetes* **1**: 87, 1952.
19. BORNSTEIN, J., and LAWRENCE, R. D.: Plasma insulin in human diabetes mellitus, *Brit. M. J.* **2**: 1541, 1951.
20. KENNY, A. J.: Unpublished data.
21. FOA, P. P.; SANTAMARIA, L.; WEINSTEIN, H. R.; BERGER, S., and SMITH, J. A. Secretion of the hyperglycemic-glycogenolytic factor in normal dogs, *Am. J. Physiol.* **171**: 32, 1952.
22. FOA, P. P.; MAGID, E. B.; GLASSMAN, M. D., and WEINSTEIN, H. R.: Anterior pituitary growth hormone and pancreatic secretion of glucagon, *Proc. Soc. Exper. Biol. & Med.* **83**: 758, 1953.
23. THOROGOOD, E., and ZIMMERMANN, B.: The effects of pancreatectomy on glycosuria and ketosis in dogs made diabetic by alloxan, *Endocrinology* **37**: 191, 1945.
24. YOUNG, F. G.: The anterior pituitary gland and diabetes mellitus, *New England J. Med.* **221**: 635, 1939.
25. DRAGSTEDT, L. R.; ALLEN, J. G., and SMITH, E. M.: Extensive insulin tolerance in diabetic dogs, *Proc. Soc. Exper. Biol. & Med.* **54**: 292, 1943.
26. RICKETTS, H. T.; BRUNSCHWIG, A., and KNOWLTON, K.: Diabetes in a totally depancreatized man, *Proc. Soc. Exper. Biol. & Med.* **58**: 254, 1945.
27. DIXON, C. F.; COMFORT, M. W.; LICHTMAN, A. L., and BENSON, R. E.: Total pancreatectomy for carcinoma of the pancreas in a diabetic person, *Arch. Surg.* **52**: 619, 1946.
28. PINCUS, I. J.: Effect of hyperglycemic-glycogenolytic factor on blood sugar of patients with diabetes, *Proc. Soc. Exper. Biol. & Med.* **74**: 377, 1950.
29. KIRTLEY, W. R.; WAIFE, S. O., and PECK, F. B.: Effect of glucagon in stable and unstable diabetic patients, *Proc. Soc. Exper. Biol. & Med.* **83**: 387, 1953.
30. KIRTLEY, W. R.; WAIFE, S. O.; HELMER, O. M., and PECK, F. B.: Effect of purified glucagon on carbohydrate and corticoid metabolism in normal and diabetic subjects, *Diabetes* **2**: 345, 1953.
31. SAKA, M. O.: Hyperglycemic-glycogenolytic factor in diabetic man and alloxan diabetic animals, *Am. J. Physiol.* **171**: 401, 1952.

QUANTITATIVE ESTIMATIONS OF ENDOMETRIAL GLYCOGEN, USING THE ANTHRONE METHOD*

H. W. PAYNE, M.D.† AND J. P. A. LATOUR, M.D.

Department of Gynecological Pathology, Royal Victoria Hospital, Montreal, Canada

GLYCOGEN is believed by many to be the direct source of nutriment for the early conceptus from the time it enters the uterine cavity to the time it is actively supported by the maternal blood stream. For this reason, efforts have been directed towards detecting abnormalities in glycogen deposition in the hope that a cause for many cases of primary sterility could be elicited. Zondek and Stein (1) stated that 18.4 per cent of the histologically normal secretory endometria in his series had a deficiency of glycogen. To this condition he attached the name of glycopenia uteri and set the lower limit of glycogen concentration in secretory endometria as 0.25 Gm. per cent. This finding stimulated research in this field and Spyker and Fidler (2), Randall and Power (3), and Laqueur (4) repeated this investigation. The series of Spyker and Fidler, in our opinion contained too few secretory endometria to yield conclusive results, but Randall and Power and Laqueur obtained values consistently higher than those of Zondek and Stein. In addition, they could find no conclusive evidence to substantiate the claim of Zondek respecting glycopenia uteri.

We first became interested in endometrial glycogen when we developed a qualitative test for secretory endometria on the basis of a significant increase in concentration of glycogen (Sabin and Latour (5)). We were impressed by the fact that none of the foregoing investigators had found a definite correlation between menstrual day and glycogen concentration in the endometrium. We attributed this to an inadequate system for dating the endometria and were of the opinion that any finite day-to-day changes could only be determined by correlation with the histologic date, as determined by the method of Hertig (6). We decided to repeat the investigation of endometrial glycogen with the view that any clarification of the cyclic changes in its concentration would be useful in the study of primary sterility.

Received for publication December 20, 1954.

* This investigation was supported by a grant from the Blanche E. Hutchison Fund.

† Research Fellow, Dept. of Gynecological Pathology, Royal Victoria Hospital. Present address: Ohio State University Hospital, Columbus, Ohio.

MATERIAL AND METHOD

Material

Ninety specimens were assayed for glycogen. Most of them were dated and examined, using formalin-fixed tissues. Suitable specimens were fixed in 80 per cent alcohol at 0° C. for twenty-four hours and in absolute alcohol for twenty-four hours at room temperature. The alcohol-fixed specimens were stained for glycogen, using periodic-acid-Schiff reagent according to the technique of Glegg, Clermont and Leblond (7). Harris haemotoxylin was used as a counter stain. Control slides were stained after the application of a solution of alpha amylase.

Specimens were obtained from patients attending the sterility and gynecology clinics and from tissues submitted to the pathology department. Hysterectomy specimens were immediately opened and the endometrium removed from the fundus by scraping with a scalpel held perpendicularly to the surface. Care was taken not to include areas of basalis or lower uterine segment. Specimens obtained from patients attending the sterility and gynecology clinics were removed with a suction curette. All specimens were immediately transferred to a piece of gauze to remove excess blood and mucus, then transferred to a previously tared test tube (10×85 mm.), and the gross weight ascertained. Thirty per cent KOH was added to the test tube in the proportion of 2 ml. per Gm. of tissue. The test tube was promptly immersed in a boiling-water bath and heated for thirty minutes. The digest was transferred to a 25-ml. volumetric flask and made up to volume with distilled water. All tissue was handled in a prompt and uniform fashion and the error due to glycogenolysis was small and constant.

Method

The photocolorimetric anthrone method of Peterson and Rose (8) was modified in the following manner:

1. A 5-ml. aliquot of the diluted digest was pipetted into a 10-ml. volumetric flask and adjusted to pH 4.
2. A 10-ml. portion of 95 per cent alcohol was added to a 5-ml. aliquot of supernatant digest and this was refrigerated for forty-five minutes.
3. The unknown solution was made up to a final volume of 10 ml.
4. The rate of addition of the 0.1 per cent anthrone solution was changed to between thirty-five and forty-five seconds while the Evelyn colorimeter tube was partially immersed in a cold water bath.
5. The reaction mixture was heated in a boiling-water bath for seven minutes, following which it was quickly cooled in a cold running-water bath for fifteen minutes.
6. The density of the developed color was determined, using a 620-mμ filter.

The accuracy of this method was thoroughly investigated and the recoveries ranged between 90 and 107 per cent. The range of values obtained agrees closely with those of Randall and Power and of Laqueur. The glycogen standard contained 5.2 per cent water (City Chemical Corporation, New York).

Grouping of results

The results are divided into three groups. In Group I (Tables 1 and 2), the specimens were obtained from patients between the ages of 20 and 35 who had normal cycles. The specimens showed normal histologic structure. In Group II (Table 3), the patients were between the ages of 35 and 45 and most of them had an abnormal menstrual history. The specimens were histologically within normal limits. In Group III something abnormal

TABLE 1. GLYCOGEN CONTENT OF ENDOMETRIA OBTAINED FROM PATIENTS BETWEEN THE AGES OF 20 AND 25 WITH NORMAL MENSTRUAL HISTORIES

Case No.	Day No.	Glycogen (Gm. %)	Case No.	Day No.	Glycogen (Gm. %)
1	early prolif.	0.133	19*	21–22	0.593
2	early prolif.	0.200	20§	21–22	0.340
3	early prolif.	0.262	21*	21–22	0.360
4	late prolif.	0.225	22	21–22	0.512
5	late prolif.	0.216	23§	21–22	0.509
6	late prolif.	0.288	24	21–22	0.400
7	16	0.685	25	21–22	0.368
8	16	0.753	26*	23	0.700
9	17	1.35	27*	23	0.744
10	17	1.36	28†	23	0.609
11†	17	1.44	29	24	0.783
12	17	1.56	30	25–26	0.653
13	18	1.22	31	25–26	0.685
14§	18	1.15	32	25–26	0.716
15	19	1.11	33	27	0.795
16	19	0.888	34†	27	1.16
17	19	0.849	35	early menstrual	0.66
18	20	no specimens	36	menstrual	0.231
			37	menstrual	0.302
			38	menstrual	0.165

* § † Multiple biopsies, same patient (monthly intervals).

TABLE 2. AVERAGE GLYCOGEN CONCENTRATIONS IN ENDOMETRIA OBTAINED FROM PATIENTS IN 20–35 AGE GROUP

Day	No. of specimens	Glycogen (Gm. %)	
		Avg.	Range
Early prolif.	3	0.200	Detectable to 0.200
Late prolif.	3	0.243	0.216 to 0.288
16	2	0.719	0.685 to 0.753
17	4	1.42	1.35 to 1.56
18	2	1.18	1.15 to 1.22
19	3	0.949	0.849 to 1.11
20	none	—	—
21–22	7	0.439	0.340 to 0.593
23	3	0.684	0.609 to 0.744
24	1	0.783	—
25–26	3	0.685	0.650 to 0.716
27	2	0.977	0.795 to 1.16

TABLE 3. AVERAGE GLYCOGEN CONCENTRATIONS IN ENDOMETRIA OBTAINED FROM PATIENTS BETWEEN THE AGES OF 35 AND 45

Day	No. of specimens	Glycogen (Gm. %)	
		Range	Avg.
Prolif.	5	0.212 to 0.280	0.253
16	3	0.420 to 0.747	0.532
17	3	0.955 to 1.60	1.29
18	2	0.812 to 1.29	1.05
20	2	0.865 to 0.791	0.848
21–22	3	0.791 to 1.19	0.913
23	2	0.648 to 0.782	0.715
24	4	0.676 to 1.16	0.825
25–26	4	0.512 to 0.723	0.654
27	1		0.714

could be established either in the clinical history of the patient or in the histologic pattern of the specimen. Most of the specimens in Group III were obtained from patients between the ages of 20 and 35 years.

RESULTS

Group I (Tables 1 and 2)

The concentration of glycogen in proliferative endometria was low and in 6 specimens did not exceed 0.288 Gm. per cent.

The concentration of glycogen rose sharply in the early secretory phase to a maximum average value of 1.42 Gm. per cent on day 17. From day 18 to day 21–22 there was a sharp decrease in glycogen concentration to an average value of 0.437 Gm. per cent. There was a moderate increase on day 23 to an average value of 0.684 Gm. per cent. This level was maintained until day 25–26, when an average value of 0.685 Gm. per cent was noted. On day 27, there was another moderate increase to an average value of 0.977 Gm. per cent. During menstruation there was a sharp decrease, the highest value of 0.66 Gm. per cent being noted at the onset of menstruation. The lowest concentration of glycogen obtained from the specimens of menstrual endometrium was less than 0.165 Gm. per cent.

Table 2 shows the average day-to-day values for Group I. Figure 1 is the resulting graph when the average values in Table 2 are plotted against the menstrual day. This graph represents the "normal" curve for glycogen concentration in the endometrium throughout the menstrual cycle. The curve showed a moderate dip on day 21–22. The fact that this "dip" in the curve was real is shown by the values obtained from specimens # 19, 21, 26 and 27, which were obtained from the same patient at monthly

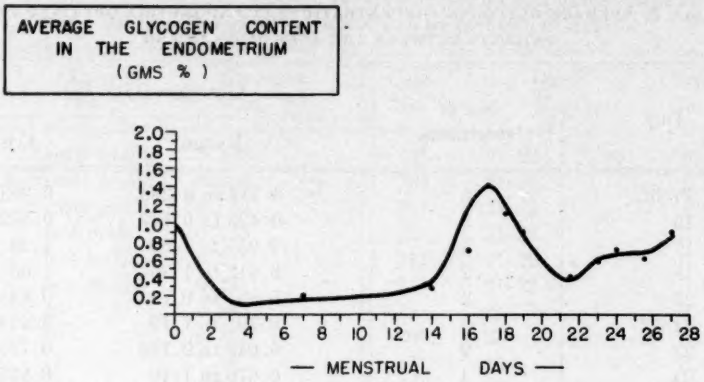

FIGURE 1

intervals. Specimens # 19 and 21, which were dated day 21–22, had less glycogen per 100 Gm. of fresh weight than specimens # 26 and 27, which were dated day 23. The findings in specimens # 14, 20 and 23 which were obtained from another patient in the day 21–22 period substantiate this point, in that they had much less glycogen per 100 Gm. of fresh weight than specimen # 14 which was dated day 18, or other specimens from the day 23–26 period. Findings in specimens # 11, 28 and 34, which were obtained from a different patient, confirm the foregoing impression.

Group II (Table 3)

The highest concentration of glycogen in endometria of the proliferative phase was 0.280 Gm. per cent. The results did not differ significantly from those in Group I.

In the secretory phase, Group II showed a greater range of values on any particular day than Group I. For instance, values on day 16 ranged from 0.420 to 0.747 Gm. per cent, and those on day 17 ranged from 0.955 to 1.60 Gm. per cent. The comparable ranges in Group I were 0.685 to 0.753, and 1.35 to 1.56 Gm. per cent, respectively. Too much emphasis should not be placed on the increased range of day 16, for in this part of the secretory phase there was a marked increase in glycogen concentration over a short period of time and a wide range was to be expected. The differences on day 17 were more pertinent, for on this day the maximum glycogen concentration was noted.

The most easily discernible difference from day 18 to day 21–22 inclusive was the lack of a sharp decrease in glycogen concentration, as was noted in the corresponding period of Group I. There was a wide range of values—from 0.791 to 1.19 Gm. per cent. We regard this observation as important and its significance will be discussed later.

From day 23 to day 25–26 inclusive, most of the values agreed with those obtained from the corresponding period in Group I. In only 1 specimen was there an excessively high value.

Group III

In 13 specimens the glycogen concentration did not correspond to the normal value for that day. In 5 of these specimens no demonstrable histologic changes from the normal could be noted, using hematoxylin and eosin stains. Two of these specimens were obtained from diabetic women—one controlled, and the other uncontrolled. The specimen from the uncontrolled diabetic had an abnormally high concentration of 1.18 Gm. per cent on day 20. The specimen from the controlled diabetic had an abnormally low concentration of 0.422 Gm. per cent on day 19.

Ten specimens in this group were classified as normal with respect to histologic picture and glycogen concentration. Four of the patients from whom these specimens were obtained had menstrual irregularities, and 5 were receiving thyroid therapy. The other specimen was one of two obtained from the same patient; the second one was diagnosed as a "mixed" endometrium.

Impressions gained through the histochemical study of glycogen in the endometrium

Only a few features could be determined by the use of periodic-acid-Schiff reagent. The cyclic changes in sites of glycogen deposition have been fully described by Moricard (9) and Arzac (10). Our studies show that an amylase-resistant secretion appears on the luminal edge of the glands on day 17 of the normal cycle and progressively increases until day 22. Thus, the increase in the amount of amylase-resistant luminal secretion and the decrease in glycogen concentration occur concomitantly. MacKay (11) has shown that this secretion is probably a mucoprotein or a mucopolysaccharide. After day 22, the secretion becomes inspissated and stains more heavily.

We were impressed by the normal variability in the sites and extent of glycogen deposition in the gland cells. Many specimens in Group I, which were normal in every respect as far as we could determine, had glands which contained little or no glycogen. In other glands, glycogen was deposited in the cells in sites different from that of the overall picture. This, and the fact that the glycogen concentration varies markedly from one part of the secretory phase to another, must be taken into consideration whenever histochemical means are employed to estimate glycogen concentration in the endometria of sterility patients.

DISCUSSION

The fact that glycogen is present in proliferative endometria indicates the diversified role that this substance has in the metabolism of the endometrium. According to Stuermer and Stein (12) anaerobic glycolysis predominates in the poorly vascularized proliferative endometrium. Proliferative endometria have a higher concentration of ribonucleic acids (13, 14) than do secretory endometria, which usually indicates a higher rate of protein synthesis. This results in an anomalous metabolic state whereby a tissue which is rapidly synthesizing protein has a relatively poor supply of energy, and may explain the relatively small concentration of glycogen in the proliferative phase. A possible explanation is that the metabolites which are normally converted into glycogen, and glycogen itself, may be utilized in essential energy-yielding reactions to compensate for the inefficiency of anaerobic glycolysis.

In the early secretory phase, there is a decrease in the amount of ribonucleic acids in the basal portion of the gland cells, concomitant with the appearance of glycogen in this position (13). During the early secretory phase, the ribonucleic acids are located in the apical portions of the gland cells along with the glycogen. Presumably, the ribonucleic acids are concerned with the synthesis of enzymatic, structural and secretory proteins. The activities of the enzymes succinic dehydrogenase and malic dehydrogenase, the rate of aerobic glycolysis, and the concentration of cytochrome c, all are increased (12). Thus the increase in glycogen concentration in the early secretory phase occurs concomitantly with the increased production of utilizable energy through the oxidation of pyruvate in the tricarboxylic-acid cycle. Since the passage of glucose into the gland cells and its storage as glycogen requires the expenditure of energy, the increased glycogen concentration may be directly attributed to the change to the aerobic phase of carbohydrate metabolism.

The morula enters the uterine cavity on day 17–18 (15) and a suitable environment for its development into the blastocyst must be maintained. This is the period of maximum secretion and although the nature of this secretion cannot be established, it is of a complex nature. The synthesis of mucoprotein, of the ribonucleic acids which are present in the luminal secretion of secretory glands as well as proliferative glands (11), and the absorption and passage of ions into the lumina of the glands, all require the expenditure of energy. It is therefore logical to assume that the rate of breakdown of glycogen would be increased to supply the necessary energy through the oxidation of pyruvate in the tricarboxylic-acid cycle. Our findings indicate that it is, and Jones (16) found indirect evidence that this occurs. Jones found the maximum adenosine triphosphatase activity on days 21-23. Although this activity cannot be attributed to any one

enzyme, there is probably increased utilization of adenosine triphosphate in various synthetic and secretory processes. Since the oxidation of pyruvate in the tricarboxylic-acid cycle gives rise indirectly to most of the adenosine triphosphate, the low concentrations of glycogen in this period are probably due to the increased rate of breakdown of glycogen to pyruvate. In addition, glycogen probably contributes to the formation of the carbohydrate moiety of the luminal mucoprotein. How much of the glycogen is released into the lumina of the glands as glucose-6-phosphate or other low-molecular unstainable intermediates cannot be established.

We regard the low concentrations of glycogen on day 21-22 as an indication of maximal secretion. As a corollary to this statement, high values in this period are indicative of diminished secretion. With this in mind, the high values obtained from specimens in Groups II and III assume added significance. A defective secretion in this period could cause the death of the blastocyst and be one of the causes of infertility in the older age group, and in a certain proportion of younger women.

The marked day-to-day changes in glycogen concentration explain the scattered values found in the secretory phase by the earlier workers. Moreover, the values obtained on day 21–22, when the difference in methods is considered, account for the glycopenia uteri of Zondek. In an evenly distributed series of secretory endometria, these low values would account for 15 per cent of the total—a figure which closely approximates the incidence of 18.4 per cent stated by Zondek.

Additional evidence to support these views is gained from Group III. In the 2 specimens obtained from uncontrolled and controlled diabetics, the results fell on opposite sides of the normal curve. It is beyond the scope of this paper to discuss the level of carbohydrate metabolism through which insulin exerts its effect, but the net effect of insulin is to cause increased oxidation and utilization of glucose. The fact that the concentration of glycogen in the day-20 specimen from the uncontrolled diabetic was abnormally high would indicate that glycogen was not being broken down to supply the energy and metabolites needed for the secretory process to proceed normally.

Little emphasis will be placed on the changes in glycogen concentration after day 23 of the normal cycle, for Brewer (17) has shown that the corpus lutem begins to degenerate at about this time. In addition, if pregnancy occurs, the conceptus at this time is almost completely imbedded (15). Moreover, it is probably nourished by the maternal blood stream through the medium of the edematous stroma or by cytolysis of the decidua until the maternal blood first makes its appearance in the lacunar spaces of the trophoblast on day 25 of the normal cycle.

Other results from Group III indicate that the glycogen concentration of

the endometrium may be normal when the patient has a history of menstrual irregularities. Since there were only 4 specimens in this subgroup, and it was impossible to establish whether the cyclic changes in glycogen concentration were taking place, a lengthy discussion is not merited. However, it is noteworthy that the glycogen concentration of the endometrium may be normal under such adverse circumstances.

COMMENT

In tissues, other than endometrium, normal synthesis of protein and fat and the maintenance of secretory processes are dependent upon the oxidation and utilization of glycolytic metabolites. The same may be said to apply in the endometrium. Thus, defective oxidation and utilization of the derivatives of glycogen may lead to an environment detrimental in many ways to the development of the blastocyst.

SUMMARY

1. The photocolorimetric anthrone method of Peterson and Rose has been adapted to endometrial glycogen estimations.

2. The concentration of glycogen is low in proliferative endometria.

3. The maximum concentration of glycogen occurs on day 17.

4. The lowest concentration in the secretory phase occurs on day 21–22.

5. The concentration of glycogen rises moderately on day 23 and remains constant until day 27.

6. On day 27 there is a further moderate increase.

7. The concentration of glycogen is low in menstrual endometria.

8. There is evidence that the cyclic changes in glycogen concentration do not occur in older women and in a small proportion of younger women.

REFERENCES

1. Zondek, B., and Stein, L.: Glycogen content of the human uterine mucosa glycopenia uteri, *Endocrinology* **27**: 395, 1940.
2. Spyker, M. A., and Fidler, R. S.: Glycogen studies on human endometrium: correlation of quantitative chemical estimation and the qualitative demonstration by histological methods, *J. Clin. Endocrinol.* **2**: 365, 1942.
3. Randall, L. M., and Power, M. H.: Amounts of glycogen in endometrium, *Proc. Staff Meet., Mayo Clin.* **17**: 158, 1942.
4. Laqueur, W.: Ueber den Glykogengehalt der Uterus-schleimhaut, *Monatschr. f. Geburtsh. u. Gynäk.* **119**: 223, 1945.
5. Sabin, M., and Latour, J. P. A.: A test for the rapid diagnosis of secretory endometrium, *Fertil. & Steril.* **5**: 44, 1954.
6. Hertig, A. T.: Diagnosing the endometrial biopsy, *in* Diagnosis in Sterility. Proc. Conference on Sterility (1945), ed. by E. T. Engle. Springfield, Ill., Charles C Thomas, Publisher, 1946, p. 93.

7. Glegg, R. E.; Clermont, Y., and Leblond, C. P.: Use of lead tetra-acetate, benzidine, o-dianisidine, and "film test" in investigating periodic-acid-Schiff technic, *Stain Technol.* **27**: 277, 1952.
8. Peterson, R., and Rose, D.: Estimation of glycogen by the use of anthrone, *Canad. J. Technol.* **29**: 317, 1951.
9. Moricard, R., and de Senarclens, F.: Change en glycogène et modifications cytologiques utérines dépendant des actions folliculiniques et progestiniques, *Compt. rend. Soc. de biol.* **141**: 57, 1947.
10. Arzac, J. P., and Blanchet, E.: Alkaline phosphatase and glycogen in human endometrium, *J. Clin. Endocrinol.* **8**: 315, 1948.
11. McKay, D. G.: Metachromasia in endometrium, *Am. J. Obst. & Gynec.* **59**: 875, 1950.
12. Stuermer, V. M., and Stein, R. J.: Cytodynamic properties of human endometrium; metabolism and enzymatic activity of human endometrium during menstrual cycle, *Am. J. Obst. & Gynec.* **63**: 359, 1952.
13. Atkinson, W. B.; Engle, E. T.; Gusberg, S. B., and Buxton, C. L.: Histochemical studies on abnormal growth of human endometrium, *Cancer* **2**: 132, 1949.
14. Stein, R. J., and Stuermer, V. M.: Cytodynamic properties of human endometrium; variations in nucleo-protein content of endometrium during menstrual cycle, *Am. J. Obst. & Gynec.* **61**: 414, 1951.
15. Hertig, A. T., and Rock, J.: Implantation and early development of human ovum, *Am. J. Obst. & Gynec.* (suppl.) 61 A: 8, 1951.
16. Jones, H. W.; Wade, R., and Goldberg, B.: Phosphate liberation by endometrium in presence of adenosinetriphosphate, *Am. J. Obst. & Gynec.* **64**: 1118, 1952.
17. Brewer, J. I.: Studies of human corpus luteum, *Am. J. Obst. and Gynec.* **44**: 1048, 1942.

SERUM PROTEIN-BOUND IODINE LEVELS FOLLOWING ADMINISTRATION OF THYROXINE IN VARIOUS DISEASES*†

T. S. DANOWSKI, M.D., R. SCHWARTZ, M.D., G. J. RHODES, M.D., R. D. YODER, M.D., A. J. PUNTERERI, M.D. AND F. M. MATEER, M.D.‡

The Department of Research Medicine, University of Pittsburgh, and the Children's, Elizabeth Steel Magee, and Veterans Hospitals, Pittsburgh, Pennsylvania.

IT IS known that serum protein-bound iodine (PBI) levels may be altered in circumstances other than hyper- or hypothyroidism (1). For example there are the increases associated with pregnancy (2–5) and muscular dystrophy (6, 7) and the decreases which may be noted in cirrhosis (8) or in subjects receiving cortisone or ACTH (9–14). It has also been reported that in patients with leukemia or lymphomata there may be an increase in the PBI level and the basal metabolic rate may be above the normal range (15–18). In none of the instances listed have the mechanisms involved in the particular PBI change been satisfactorily identified. It is nonetheless obvious that in these clinical and experimental situations new steady states have been established involving possible alterations in the production or release of thyroxine by the thyroid gland, the binding of thyroxine by serum proteins, the distribution of thyroxine in body fluids, or the subsequent removal of thyroxine from the proteins or from the circulation, alone or in combination. The sodium salt of *l*-thyroxine[1] has therefore been given by mouth in such conditions, in an attempt to establish the participation of these components, if any, in the serum protein-bound iodine changes.

Such administration of enough thyroxine results in a rise and a subsequent fall in the level of serum PBI. The degree of the rise and the rate of fall can be taken to reflect the absorption and subsequent fate of thyroxine.

* Presented at the Annual Meeting of the American Goiter Association, Oklahoma City, Okla., April 28–30, 1955.

This article will be included in the bound volume of the 1955 "Transactions of the American Goiter Association" published by Charles C Thomas, Publisher, which will be available early in 1956.

† Aided by grants from the United States Public Health Service, Atomic Energy Commission, and Smith, Kline, French Foundation.

‡ Established Investigator of the American Heart Association, Inc.

[1] Generously supplied by Smith, Kline and French as "Elthrin."

Since exogenous thyroid or thyroxine suppresses endogenous thyroid function (19, 20) the serial changes in serum PBI following the ingestion of thyroxine will depend upon the absorption of thyroxine, its binding by the proteins of serum, the distribution in body fluid compartments and the subsequent disruption or removal of this combination from the circulation by utilization, degradation or excretion (21–24). Though not directly established, it is probable that following a single dose the recorded peaks of the PBI change taken at a fixed time shortly after thyroxine ingestion reflect predominantly absorption binding, and an early volume of distribution or dilution of the administered thyroxine, whereas the subsequent fall depends predominantly on the rates of dissociation or removal of this complex. Obviously, in repeated dosage studies all of these factors will be intermingled save at the beginning and the end of thyroxine administration.

Studies based on this technique and reported here have revealed no abnormalities in the serum PBI response to thyroxine in pregnancy, muscular dystrophy, or as a result of steroid administration to diabetic subjects. It is possible that in Laennec's cirrhosis the response was greater than in normal subjects. On the other hand, clearly abnormal responses were observed frequently, though not invariably, in patients hospitalized with neoplastic or other diseases.

MATERIALS AND METHODS

L-thyroxine was given by mouth as the sodium salt to healthy subjects and to patients in accordance with one of several schedules: a) in single dosage on a per kilo of body weight basis, or b) in fixed amounts without regard for body mass, in single or multiple dosage. The clinical features of the patients receiving certain steroids, pregnant, or ill with cirrhosis, muscular dystrophy, lymphomata or other neoplastic or non-neoplastic diseases are tabulated. Serum was analyzed for protein-bound iodine by the method of Barker as used in this laboratory (4, 5, 20, 24). The PBI responses to thyroxine in these patients and in control subjects are illustrated, and the significance of any differences established by conventional statistical methods.

RESULTS

A. *In cirrhosis*

The patients are listed in Table 1. Pertinent laboratory findings together with the results of examination of available liver biopsy specimens are also listed. All but 3 of the patients received 0.6 mg. of sodium *l*-thyroxine each day for a total of nine days; in the remainder the dosage was based on the body weight and consisted of 0.2 mg. per Kg. given as a single dose. Control observations at these two dosage levels were obtained in 11 and 13 healthy adults, respectively.

The daily ingestion of 0.6 mg. of sodium *l*-thyroxine for nine days induced rates of rise and peak values of PBI in patients with cirrhosis which appear greater than those recorded in healthy young adults (Fig. 1). The difference in the slope of the rise in the two groups is largely attributable however to the lower pretreatment levels present in cirrhosis—4.9 gamma per 100 cc. in contrast to 5.9 gamma in the controls, with a "p" value of 0.036. The rise observed in the cirrhosis patients at the end of nine days

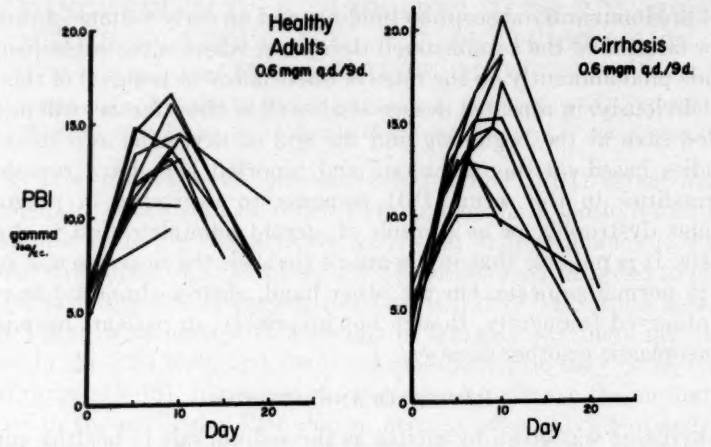

FIG. 1. *PBI changes following oral thyroxine in healthy adults and patients with cirrhosis.*

The steeper slope of rise in the group with cirrhosis is a reflection of the lower pretreatment value.

was $+9.3 \pm 3.4$ gamma per 100 cc. versus $+7.6 \pm 2.4$ gamma in the control group. The difference between these two mean values is not statistically significant.

In the per-kilogram-body-weight studies (Fig. 2) the patients with cirrhosis manifested changes in the serum PBI level which were within the range found in healthy controls.

B. *In diabetic children receiving steroids*[2]

The ages, duration of diabetes and the diet and insulin requirements of the diabetic children who were subjects of this study are listed in Table 2. The pretreatment serum PBI values fell within the range characteristic of the nondiabetic healthy child (25). From the table it is further apparent that oral sodium thyroxine administration (1.2 mg. daily for nine days) did

[2] Kindly supplied by Dr. I. V. Sollins of Chemical Specialties, Inc.

TABLE 1. EFFECT OF SODIUM-L-THYROXINE UPON SERUM PBI LEVEL OF PATIENTS WITH CIRRHOSIS

Patient Age-Sex	Liver biopsy findings	BSP liver function test (%, 45 min.)	Serum (before R_x)									Thyroxine dosage
			Alb. Glob. (Gm. %)	Cholesterol		Prothrombin		Thymol. turbid. (units)	Ict. index	Bilirubin (mg./100 cc.)	Cephflocc. (24–48)	PBI (γ/100 cc.)	
				Total (mg./100 cc.)	Esters (%)	Patient (sec.)	Control (sec.)						
O.B. 36 M	Portal cirrhosis	40	3.8/2.7			14	12	4.5		2.4	1+ 2+	4.7	0.2 mg./Kg., 1x
C.M. 44 M	Portal cirrhosis	32	1.8/4.1	265	50	16	12	6.6			2+ 4+	8.2	0.2 mg./Kg., 1x
J.P. 61 M	No biopsy	40	3.9/2.4	170	52	16	12	13		2.3	3+ 4+	4.6	0.2 mg./Kg., 1x
W.M. 44 M	Portal cirrhosis	10	5.0/1.5			20	14		26		3+ 4+	3.1	0.6 mg., q.d./9d.
C.F. 38 M	No biopsy	38	3.7/3.4	239	44	16	14	11	36		1+ 1+	4.8	0.6 mg., q.d./9d.
S.S. 40 M	Portal cirrhosis	36	3.4/3.3	163	60	19	15		18	13.4+	2+ 3+	5.0	0.6 mg., q.d./9d.
S.B. 30 M	Portal cirrhosis	5	4.7/2.8			16	16	4		.94	0 1+	5.5	0.6 mg., q.d./9d.
R.B. 45 M	Portal cirrhosis (autopsy)	40	1.3/4.5	282		14	12			0.75	4+ 4+	5.6	0.6 mg., q.d./9d.
E.B. 58 F	Biliary cirrhosis		2.9/3.4	355		13	12	5.6	18		4+ 4+	6.9	0.6 mg., q.d./9d.
E.K. 42 M	Portal cirrhosis	10	5.0/3.3	360	55	16	14		9		2+ 3+	5.6	0.6 mg., q.d./9d.
J.T. M	Laennec's cirrhosis: clinical and laboratory data not available											3.2	0.6 mg., q.d./9d.
D.M. M	Laennec's cirrhosis: clinical and laboratory data not available											5.0	0.6 mg., q.d./9d.
R.S. M	Laennec's cirrhosis: clinical and laboratory data not available											4.5	0.6 mg., q.d./9d.

not increase the insulin requirement and neither did the combination of thyroxine and Reichstein's compound S (Δ^4-pregnene-17α,21-diol-3,20-dione) when the latter was given intramuscularly at a dosage level of 50 mg. four times each day for nine days. On the other hand, the administration of compound F (hydrocortisone) or compound L (allopregnan-3β,-17α,diol-20-one) intramuscularly in conjunction with oral thyroxine was associated with a distinct increase in the insulin requirement. These findings are cited in order to indicate when the compounds were or were not exerting physiologic effects.

TABLE 2. DIABETIC CHILDREN RECEIVING SODIUM SALT OF L-THYROXINE 1.2 MG. DAILY FOR 9 DAYS, WITH AND WITHOUT CONCOMITANT ADMINISTRATION OF COMPOUND S, F, OR L

Subject	Age	Sex	Body wt. (Kg.)	Duration of diabetes	Diet		Insulin dosage*	Serum PBI before R_x (γ/100 cc.)	Therapy	
					P.F.C.	Calories			Thyroxine	Steroid
R.K.	16	M	61.8	5 yr. 3 mo.	122-125-260	2631	P18R34→P16R38	3.5	1.2 mg./q.d./9d.	—
K.R.	15	M	61.8	3 yr. 10 mo.	115-125-330	2005	P18R54→P18R56	6.1	1.2 mg./q.d./9d.	—
R.S.	13	F	44.8	4 yr. 1 mo.	172-158-461	3954	P27R60→P27R70	5.6	1.2 mg./q.d./9d.	—
R.V.S.	14	M	52.0	3 yr.	135-145-275	2955	NPH62R6→NPH74R6	7.9	1.2 mg./q.d./9d.	—
C.L.	11	F	34.2	6 yr. 4 mo.	120-100-250	2380	P10R28→P10R36	5.6	1.2 mg./q.d./9d.	Cpd S, 200 mg./q.d./9d.
R.C.	15	M	59.6	13 yr. 5 mo.	135-110-300	2730	P12R54→P14R56	4.1	1.2 mg./q.d./9d.	Cpd S, 200 mg./q.d./9d.
G.P.	15	M	43.2	5 yr. 1 mo.	120-100-250	2380	P10R27→P12R30	4.3	1.2 mg./q.d./9d.	Cpd S, 200 mg./q.d./9d.
J.B.	13	M	31.6	7 yr.	125-100-275	2500	P16R28→P30R56	4.6	1.2 mg./q.d.99d.	Cpd F, 200 mg./q.d./9d.
D.McA.	16	F	58.2	2 yr.	75-115-150	1935	P22R55→P36R82	2.8	1.2 mg./q.d./9d.	Cpd F, 200 mg./q.d./9d.
J.D.	13	F	48.8	3 yr.	125-135-260	2755	P18R42→P22R75	3.8	1.2 mg./q.d./9d.	Cpd L, 200 mg./q.d./9d.

* P=protamine. R=regular.

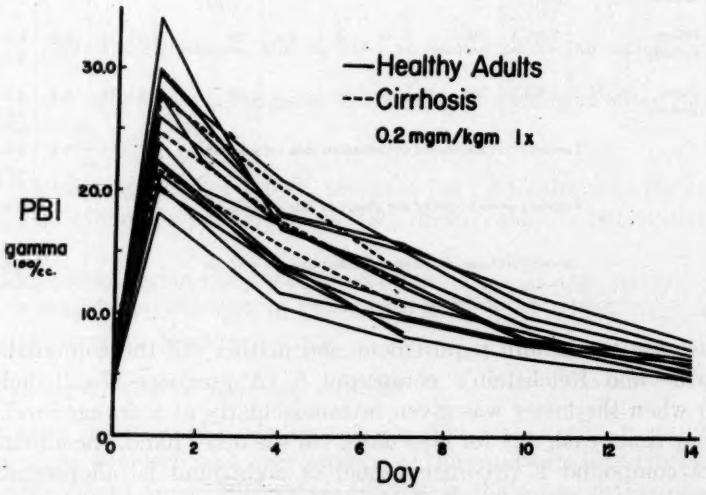

FIG. 2. *Serum PBI changes following single doses of thyroxine administered to healthy adults and to patients with Laennec's cirrhosis.*

The response in the 3 patients with cirrhosis (broken lines) was of the same order of magnitude as in the adult controls (solid lines).

From Figure 3 it is readily apparent that neither compound F, compound S nor compound L modified the rise in protein-bound iodine concentration induced by thyroxine ingestion. It should be pointed out that control studies in healthy children are not available to establish whether the diabetic responds to thyroxine as does the nondiabetic. Our observations do

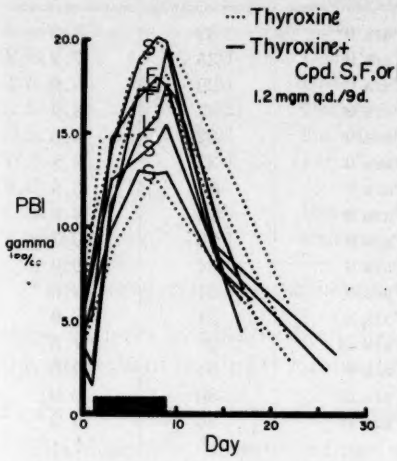

FIG. 3. *Absence of a discernible effect of steroids upon serum PBI changes following the oral administration of thyroxine to diabetic children.*

The rate of rise to the peak reached at nine days and the subsequent decline were the same in the two groups of diabetic children.

show, however, that this response in the diabetic was not discernibly modified by the steroids tested.

C. *In pregnancy*

The first 10 subjects in this group (Table 3) were all inmates of a Salvation Army home for pregnant girls; the remainder were regular visitants to the obstetrical clinic of the Elizabeth Steel Magee Hospital. In each instance gestation ended in a viable birth.

From Figure 4 it is apparent that prior to the administration of sodium *l*-thyroxine the protein-bound iodine levels were higher in the pregnant women than in the healthy young adult control subjects. This is in keeping with previous studies in this and in other laboratories (2–5). A single dose of sodium *l*-thyroxine, (5.4 mg. orally) caused equal increments in the 24-hour serum PBI level—$+9.5 \pm 2.3$ gamma per 100 cc. in the healthy adults and $+10.2 \pm 1.8$ gamma in the pregnant women. The difference between these means is not statistically significant. The differences in the actual values at twenty-four hours closely approximated those present in the two groups prior to therapy.

The subsequent decreases in PBI concentration developed at the same rate even though at the end of the observation period the pretreatment differences between the two groups were still evident.

TABLE 3. PREGNANT WOMEN WHO RECEIVED SODIUM L-THYROXINE *orally*—5.4 MG. IN A SINGLE DOSE

Subject	Age	Obstetrical history	Duration of pregnancy (wks.)	Serum PBI before R_X (γ/100 cc.)
B.	21	Grav. i Para 0	32	8.2
C.L.	16	Grav. i Para 0	35	7.2
C.	20	Grav. i Para 0	29	8.9
E.	19	Grav. i Para 0	32	8.9
J.	19	Grav. i Para 0	32	8.6
L.	23	Grav. i Para 0	32	8.8
M.	33	Grav. i Para 0	36	6.8
R.	18	Grav. i Para 0	12	8.0
P.	20	Grav. i Para 0	26	10.7
M.	21	Grav. i Para 0	34	9.9
M.B.	19	Grav. i Para 0	31	8.6
M.D.	21	Grav. iii Para ii	34	8.9
D.G.	34	Grav. iv Para iii	28	7.5
E.G.	15	Grav. i Para 0	34	9.6
C.G.	28	Grav. iv Para iii	30	6.9
V.H.		Grav. v Para iv	34	6.9
B.K.				7.1
L.L.	27	Grav. iv Para ii	34	7.5
P.M.	24		26	8.2
R.M.	19	Grav. iii Para ii	34	8.4
E.P.		Grav. v Para ii	26	7.4
M.P.	21	Grav. v Para iii	34	8.7
D.S.	33	Grav. iv Para ii	30	7.9
L.V.	21	Grav. i	22	9.0
O.W.	35	Grav. iv Para iii	35	8.4

The identity of the responses in the two groups is readily demonstrated by superimposing the two sets of curves at a common point of origin.

D. *In muscular dystrophy*

The children participating in this study are described in Table 4. The higher protein-bound iodine levels referred to earlier as usually present in muscular dystrophy were evident prior to thyroxine therapy in this small group of children afflicted with the disease (Table 4). The mean value of 5.8 gamma per 100 cc. is higher than the average for the few healthy children shown in this figure or for the larger published series (4.7 ± 0.8 gamma) from this laboratory (25).

The 24-hour values of PBI following the administration of 0.2 mg. of thyroxine *orally* per kilogram of body weight to healthy children and to

TABLE 4. CHILDREN WITH MUSCULAR DYSTROPHY WHO RECEIVED 0.2 MG. IN A SINGLE DOSE OF SODIUM L-THYROXINE PER KG. OF BODY WEIGHT

Patient	Age	Sex	Muscle changes (biopsy)	Muscle strength* (% normal)	Serum PBI before R_x (γ/100 cc.)	Total dose of thyroxine (mg.)
R.G.	11	M	Definite	Reduced	6.0	6.0
A.K.	14	M	Definite	Reduced	5.7	4.8
G.Z.	8	M	Definite	50	5.8	7.0
H.S.	9	M	Far advanced	45	5.9	5,0
W.S.	8	M	Far advanced	45	6.3	4.5
B.H.	11	F	—	70	5.3	8.0
D.H.	7	F	Definite	85	5.5	4.6
E.McM.	12	M	Definite	Reduced	5.1	6.2

* Scale based on function against gravity and against resistance.

these patients overlap without discernible segregation; this is also true of the subsequent decline in circulating PBI (Fig. 5).

E. *In patients with neoplastic diseases*

The diagnosis, biopsy findings, current therapy, and other pertinent data in the patients hospitalized with neoplastic disease and participating in this study are given in Tables 5 and 6. Four other patients with miscel-

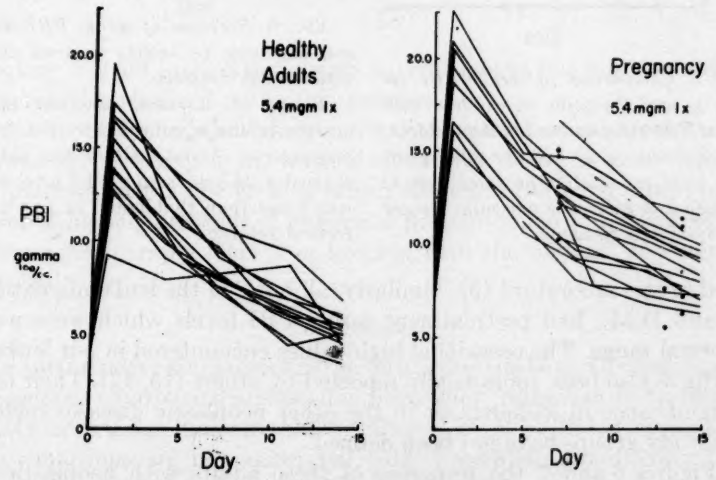

FIG. 4. *The effect of oral thyroxine upon the PBI level in pregnancy.*
At the end of the period of observation the higher PBI values in the pregnant group reflected the higher pretreatment average.

laneous diseases whose responses are depicted in the figures were hospitalized with pneumonia, tuberculosis, brain atrophy and myocardial infarction.

It is to be noted from Tables 5 and 6 that the prethyroxine values for serum PBI were not generally elevated in the patients ill with neoplastic diseases other than leukemia—average, 5.66 gamma per 100 cc. compared with a mean value of 5.44 ± 0.82 gamma in a larger series of healthy adults

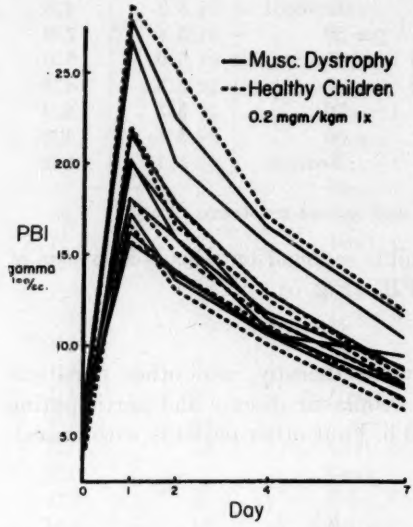

FIG. 5. *Comparison of serum PBI responses to oral thyroxine in children with muscular dystrophy and in healthy children.*

The 24-hour values and the subsequent decline were essentially the same despite the presence of extensive muscular disease in the dystrophy patients.

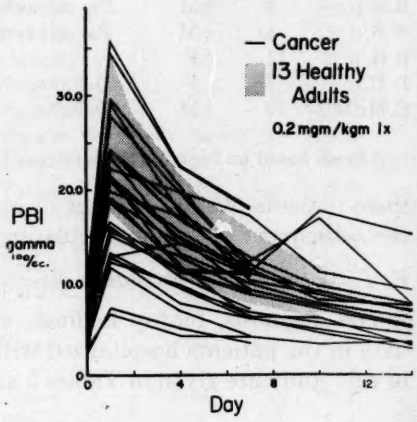

FIG. 6. *Response of serum PBI level to oral thyroxine in healthy controls and in patients with neoplasia.*

Range of response obtained in the healthy adults is indicated by the shaded background. About half of the patients showed a 24-hour serum PBI level which was lower than that noted in any of the control subjects.

studied in this laboratory (5). Similarly, all but 2 of the leukemia patients, A.D. and D.M., had pretreatment serum PBI levels which were within the normal range. The occasional high values encountered in our leukemia group have also been sporadically reported by others (15, 17). Their origin and significance in leukemia or in the other neoplastic diseases included in our study groups have not been defined.

In Figures 6 and 7 the responses of these adults with neoplastic and non-neoplastic diseases are shown against a background of control observations. It is evident that many of the cancer patients did not show a rise in

serum protein-bound iodine concentration as great as that seen in the control subjects, irrespective of whether the fixed or the per-kilogram dosage was given. This is also apparent in the mean values at twenty-four hours (Table 7). The rates of return of the PBI to pretreatment levels in the sera of these patients with neoplastic diseases appear to be comparable to those in the control subjects after the PBI had dropped to the same levels. However, this is only a visual impression.

From Figure 7 it is obvious that a lesser PBI response to exogenous

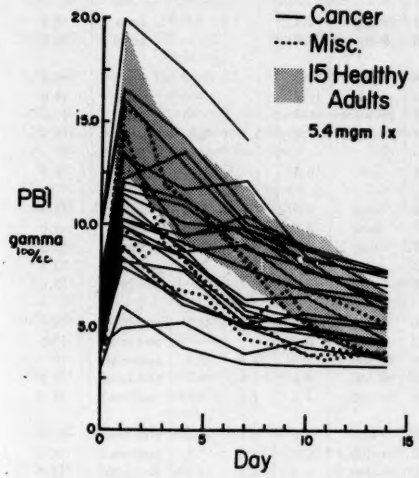

FIG. 7. *Oral thyroxine and the serum PBI response in neoplastic and in miscellaneous diseases compared to the response in healthy adults.*

Sodium *l*-thyroxine in a single dose at zero time induced much less response in patients with neoplastic and miscellaneous diseases (solid and broken lines, respectively) than in healthy controls (shaded background).

thyroxine was not limited to patients with neoplasia but was also observed in patients hospitalized with other diseases.

We were unable to relate the abnormal responses to concurrent therapy, the presence of fever, or the level of the BMR. There is some indication that patients with a low level of serum albumin or of total serum protein showed a higher incidence of abnormal protein-bound iodine values prior to thyroxine therapy. This is in keeping with the findings of Peters and Man (26).

DISCUSSION

The quantitative similarities in the serum protein-bound iodine responses to exogenous thyroxine manifested in pregnancy, muscular dystrophy, and possibly in cirrhosis indicate that the altered levels of PBI often present in these conditions are not associated with discernible abnormality in the absorption, protein-binding, and distribution of thyroxine, or in its subsequent dissociation, degradation and excretion. Hence the high level of

TABLE 5. STATUS OF ADULTS WITH NEOPLASTIC DISEASES GIVEN 0.2 MG. OF THYROXINE PER KG. OF BODY WEIGHT IN A SINGLE DOSE, *orally*

Patient; age (yrs.)	Duration of illness	Diagnosis	Sites	Body wt. change (lbs.)	Fever (degrees F.)	Serum (before R_x)			Therapy	Total dose of thyroxine (mg.)
						PBI (γ/100 cc.)	Alb. (Gm. %)	Glob. (Gm. %)		
R.C.* 28	4 mo.	Chr. myel. leukemia	Spleen	140→135	99–99.8	6.1	4.3	2.3	none	12.2
J.H. 37	2 yr.	Malig. melanoma	Nodes	155→132	none	3.6	4.3	2.0	none	12.0
J.H. 37	2 yr.	Malig. melanoma with metast.	Nodes	155→132	99–103	2.3	4.3	2.0	none	12.0
C.M. 52	40 mo.	Fibrosarcoma	L. leg, lungs	145→112	98–102	5.1			none	10.2
J.B. 33	6 yr.	Lymphosarcoma; tuberculosis	Nodes	224→200	98–99.4	7.7			none	20.0
P.C. 28	9 mo.	Malig. melanoma	Trunk, nodes	200→198	none	10.5			none	18.2
A.D. 48	6 mo.	Myel. leukemia	Nodes	178→143	none	11.6			none	13.0
A.R. 30	1 mo.	Hodgkin's	Nodes, spleen	133→121	99–101.5	5.3			none	11.0
C.D. 58	20 mo.	Chr. lymph. leukemia	Spleen, liver, nodes	153→153	98–100.2	7.5	5.1	0.6	x-ray	13.9
W.M. 22	9 yr.	Giant follic. lymphoma	Nodes	138→136	none	6.3			x-ray	13.4
C.D. 57	6 mo.	Lymphoma	Gen'd**	195→158	none	6.5	4.4	2.0	x-ray	14.4
G.H. 57	4 mo.	Retic.-cell sarcoma	Nodes	154→154	none	5.6	4.7	2.0	x-ray	14.0
E.Z. 30	6 yr.	Hodgkin's	Spleen, nodes	?→134	none	4.8			x-ray	12.2
J.D. 22	4 yr.	Multiple myeloma	Skeleton	?→133	none	5.5	4.1	2.1	post-irrad.	12.1
E.H. 64	4 yr.	Chr. lymph. leukemia	Nodes	155→150	none	6.1			post-irrad.	13.6
L.C. 38	8 yr.	Hodgkin's	Nodes	180→200	none	7.3	5.3	0.5	post-irrad.	18.2
S.D. 55	5 yr.	Lymphosarcoma	Gen'd; liver	150→132	none	3.8			post-irrad.	12.0
J.W. 54	6 yr.	Retic.-cell sarcoma	Gen'd	145→110	98–100.6	3.9	3.6	2.4	post-irrad.	10
C.M.* 25	7 yr.	Hodgkin's	Gen'd	172→120	99–100	3.5	3.8	2.7	post-irrad.	10.9
F.H.* 57	3 yr.	Chr. lymph. leukemia	Spleen, liver, nodes	185→169	99–102	4.5	4.3	1.8	post-irrad.	15.4
S.A.* 43	13 mo.	Hodgkin's	Nodes	177→165	none	5.0	4.1	2.2	post-irrad.	15
A.W.* 26	3½ yr.	Hodgkin's	Spleen, nodes	130→125	98–102.4	2.6	3.6	1.3	post-irrad.	11.4
N.M. 31	1 mo.	Retic.-cell sarcoma	Nodes	?→156	99–100.6	7.4	4.2	1.6	post-irrad.	14.2
W.K. 40	6 yr.	Lymphosarcoma	Gen'd	135→107	99–101	3.4			cpd E	8.1
R.D.* 66	2 yr.	Chr.+acute lymph. leukemia	Spleen	175→150	none	5.7			cpd E	13.6
F.B.* 35	2 yr.	Acute myel. leukemia, RHD	Spleen	144→134	none	5.2			cpd E	12.2
R.M.* 58	8 mo.	Neph. syndrome+ mult. myeloma	Skeleton	175→136	none	10.5	4.7	1.6	urethane	13.4
E.W.* 65	13 mo.	Mult. myeloma	Skeleton	162→143	none	4.8			urethane	13
F.R.* 55	4 mo.	Mult. myeloma	Skeleton	175→131	99–101	6.6	3.5	3.9	urethane	10.9
H.H. 47		Subacute leukemia	Nodes	?→147		6.8			urethane	13.4
W.J. 57	16 mo.	Chr. lymph. leukemia, lues, silicosis	Nodes	152→150	none	4.9			P^{32} in past, none current	13.6

* These patients had anemia.
** Generalised.

serum PBI in pregnancy and in muscular dystrophy and the low level often seen in cirrhosis represent changes in the steady state without any alterations in the absorption, fate and excretion of thyroxine detectable by means of these tolerance procedures. This was true in our cases even though marked impairment of liver function was present in the cirrhotic patients, and though the muscle mass was diseased in muscular dystrophy—two tissues

TABLE 6. STATUS OF PATIENTS WITH NEOPLASTIC DISEASES RECEIVING 5.4 MG. OF THYROXINE *orally* IN A SINGLE DOSE

Patient age (yrs.)	Duration of illness	Diagnosis	Sites	Body wt. change (lbs.)	Fever (degrees F.)	BMR (%)	Serum (before R_x)			Therapy	Comment
							PBI (γ/100 cc.)	Alb. (Gm. %)	Glob. (Gm. %)		
H.L. 28	5 yr.	Sarcoidosis	Gen'd*	170→156	99–100	+14	16.1**	3.9	2.4	none	
W.G. 24	2½ mo.	Hodgkin's	Nodes, spleen	208→205	none	+14	6.7	4.5	2.0	none	
C.S. 44	6 mo.	Lymphosarcoma	Nodes, spleen	?→190	none	+17	5.2			none	
O.S. 47	2 mo.	Lymphosarcoma	Nodes	157→149	none		4.3	3.5	0.7	none	
D.S. 30	17 mo.	Embryonal tumor	Nodes, chest	210→184	none	−18	6.7	4.5	2.4	none	
D.M. 27	10 mo.	Chr. myel. leukemia	Spleen, liver	140→127	97–99.4	+11	10.1			x-ray	Anemia
L.Mc. 55	5 yr.	Retic.-cell sarcoma	Spleen, gen'd	138→120	none		5.8	3.6	3.9	x-ray	
J.W. 61	3 yr.	Lymphosarcoma	Spleen, nodes	197→140	none		10.2	3.6	1.5	x-ray	Anemia
J.D. 22	4 yr.	Mult. myeloma	Skeleton	?→160	none		4.2	4.0	2.2	x-ray	
J.B. 63	2 yr.	Lymphosarcoma +leukemia	Gen'd	170→157	none	+24	6.7			x-ray	
J.D. 63	27 mo.	Lymphosarcoma	Gen'd	148→111	99–102		7.8	4.0	1.8	x-ray	Anemia
J.G. 61	15 mo.	Malig. lymphoma	Gen'd	175→146	99–102		4.7			x-ray	
C.J. 60	3 yr.	Lymphosarcoma	Gen'd	153→131	none	+23	7.0			x-ray +P^{32}	
S.K. 51	8 mo.	Mult. myeloma	Skeleton	?→158	none	+12	10.3	4.2	2.1	x-ray	
W.K. 40	6 yr.	Lymphosarcoma	Gen'd	135→107	99–101		3.0	3.6	1.2	cpd E	
G.G. 63	11 mo.	Chr. lymph. leukemia	Spleen, nodes	155→128	98–101	+52	4.2	3.2	5.6	cpd E+ testosterone	
J.H. 54	3 yr.	Lymphosarcoma; acq. hemol. anemia	Gen'd	220→187	none	+ 4	4.3			cpd E	Anemia
H.M. 58	26 mo.	Lymphosarcoma	Chest, abdomen	170→145	none	− 1	4.5			cpd E x-ray	Anemia
W.B. 23	3 yr.	Hodgkin's	Nodes	175→129	none		4.5	2.5	2.0	TEM	Anemia
W.S. 29	27 mo.	Hodgkin's	Chest, nodes	145→134	99–101	+ 1	4.6			TEM	Anemia
E.B. 35	12 mo.	Hodgkin's	Spleen, nodes	180→163	100–103		5.1			N mustard	
L.W. 27	2 yr.	Giant. follic. lymphoma	Liver, spleen, lungs	160→120	99–100	+ 1	5.1			N mustard	Anemia
J.M. 51	19 mo.	Mult. myeloma +schizophrenia	Skeleton	?→140	none		4.0	3.3	2.6	urethane	
F.Y. 49	26 mo.	Mult. myeloma, tuberculosis	Skeleton	200→183	none	−18	6.5	2.4	7.4	urethane	Uremia, anemia
L.S. 23	1 yr.	Hodgkin's	Spleen, nodes, liver	170→135	101–104		4.3	3.7	2.1	transfusion	Anemia

* Generalized.

** Review of record revealed i.v. pyelography 4 days earlier as possible explanation for high PBI level.

in which the bulk of thyroxine is metabolized. This suggests that other tissues make up for these defects or that "reverse thyroids" (*i.e.*, the salivary glands (27–29)) are more important than the liver and muscles in the disposal of excesses of thyroxine.

Neither is there any evidence that hydrocortisone (compound F) or the other steroids tested (compounds S and L) interfered with the metabolism of orally administered thyroxine in diabetic children. Hence our findings

are in keeping with previous reports that oxysteroids do not affect the peripheral disposal of thyroxine (30–33), and they differ with the sole dissenting report of Bondy (34). It is to be noted, however, that control data are not available to decide whether such children handle thyroxine in the same way as do nondiabetic children.

On the other hand the failure uniformly to induce increases in serum protein-bound iodine levels in patients hospitalized with neoplastic or other diseases, comparable to increases observed in control subjects,

TABLE 7. EFFECT OF EXOGENOUS THYROXINE ON SERUM PBI LEVEL IN PATIENTS WITH NEOPLASTIC DISEASES

Subjects	Thyroxine	Serum PBI at 24 hrs., Mean and S.D. (γ/100 cc.)
Patients	5.4 mg. 1×	11.7±4.2 [27]*
Controls	5.4 mg. 1×	14.6±7.4 [12]
Patients	0.2 mg./Kg. 1×	20.0±6.3 [17]*
Controls	0.2 mg./Kg. 1×	25.4±3.5 [10]

* The figures in brackets signify the number of determinations. Differences are statistically significant; "p" less than 0.03.

points to an abnormality of thyroxine metabolism in one or more of the facets cited, *i.e.*, in absorption, binding, distribution, dissociation, degradation or excretion. The relationship, if any, of this abnormality to the higher BMR values present in leukemia and lymphomata is to be determined.

SUMMARY AND CONCLUSION

The rise and the subsequent fall in serum protein-bound iodine concentration following administration of thyroxine is of the same general order of magnitude in 1) control subjects, 2) diabetic children receiving steroid therapy, 3) pregnancy, 4) muscular dystrophy, and 5) some cases of cirrhosis. In adult patients with neoplastic diseases, sodium *l*-thyroxine given either on a body-weight or on a fixed dosage basis resulted in a rise in serum protein-bound iodine concentration which was often less than that elicited in healthy controls. Similar responses were also seen in a few patients with non-neoplastic diseases. These findings point to an unidentified abnormality in the absorption, protein binding, distribution, dissociation, degradation or excretion of this amino acid.

REFERENCES

1. DANOWSKI, T. S.: Interrelations of thyroid and iodine metabolism. Harvey Grant Beck Memorial Lecture. *Maryland M. J.* **2**: 530–536 (Oct.) 1953.

2. HEINEMANN, M.; JOHNSON, C. E., and MAN, E. B.: Serum precipitable iodine concentrations during pregnancy, *J. Clin. Invest.* **27**: 91–97 (Jan.) 1948.
3. MAN, E. B.; HEINEMANN, M.; JOHNSON, C. E.; LEARY, D. C., and PETERS, J. P.: The precipitable iodine of serum in normal pregnancy and its relation to abortions, *J. Clin. Invest.* **30**: 137–150 (Feb.) 1951.
4. DANOWSKI, T. S.; GOW, R. C.; MATEER, F. M.; EVERHART, W. C.; JOHNSTON, S. Y., and GREENMAN, J. H.: Increases in serum thyroxin during uncomplicated pregnancy, *Proc. Soc. Exper. Biol. & Med.* **74**: 323–326 (June) 1950.
5. DANOWSKI, T. S.; HUFF, S. J.; MRVOS, D.; WIRTH, P.; GEORGE, R. S., and MATEER, F. M.: Is pregnancy followed by relative hypothyroidism? *Am. J. Obst. & Gynec.* **65**: 77–80 (Jan.) 1953.
6. DANOWSKI, T. S.: Electrolyte and endocrine studies in muscular dystrophy, *Am. J. Phys. Med.* **35**: 1955.
7. DANOWSKI, T. S.; LEINBERGER, M.; WIRTH, P., and GREENMAN, L.: Endocrine studies in muscular dystrophy (in preparation).
8. KYDD, D. M., and MAN, E. B.: Precipitable iodine of serum (SPI) in disorders of the liver, *J. Clin. Invest.* **30**: 874–878 (Aug.) 1951.
9. WOLFSON, W. Q.; BEIERWALTES, W. H.; ROBINSON, W. D.; DUFF, I. F.; JONES, J. R.; KNORPP, C. T., and EYA, M.: Corticogenic hypothyroidism: its regular occurrence and clinical significance during prolonged therapeutic administration of ACTH or cortisone, *J. Lab. & Clin. Med.* **36**: 1005–1006 (Dec.) 1950.
10. WOLFSON, W.; BEIERWALTES, W. H.; ROBINSON, W. D.; DUFF, I. F.; JONES, J. R.; KNORPP, C. T.; SIEMIENSKI, J. S., and EYA, M.: Corticogenic hypothyroidism: its incidence, clinical significance and management during prolonged treatment with ACTH or cortisone, *in* Proceedings of Second Clinical ACTH Conference, ed. by J. R. Mote. Philadelphia, The Blakiston Co., 1951, vol. 2, pp. 95–122.
11. HILL, S. R., JR.; REISS, R. S.; FORSHAM, P. H., and THORN, G. W.: The effect of adrenocorticotropin and cortisone on thyroid function: thyroid-adrenocortical interrelationships, *J. Clin. Endocrinol.* **10**: 1375–1400 (Nov.) 1950.
12. HARDY, J. D.; RIEGEL, C., and ERISMAN, E. P.: Experience with protein-bound iodine (PBI); the effect of ACTH and cortisone on thyroid function, *Am. J. M. Sc.* **220**: 290–292 (Sept.) 1950.
13. FOSTER, W. C., and MCCLENDON, J. F.: Effect of cortisone on tissue protein-bound iodine and iodine content of thyroid gland, *Fed. Proc.* **11**: 48 (March) 1952.
14. FREDRICKSON, D. S.; FORSHAM, P. H., and THORN, G. W.: The effect of massive cortisone therapy on measurements of thyroid function, *J. Clin. Endocrinol. & Metab.* **12**: 541–553 (May) 1952.
15. CURTIS, G. M.; DAVIS, C. B., and PHILLIPS, F. J.: Significance of the iodine content of human blood, *J.A.M.A.* **101**: 901–905 (Sept.) 1933.
16. STEVENS, C. D.: Determination of iodine in biological materials; simplified technique, *J. Lab. & Clin. Med.* **22**: 1076–1079 (July) 1937.
17. TURNER, K. B.; DELAMATER, A., and PROVINCE, W. D.: Observations in the blood iodine; blood iodine in health, in thyroid and cardiorenal disease and in leukemia, *J. Clin. Invest.* **19**: 515–524 (May) 1940.
18. PERRY, W. F., and COSGROVE, J. B. R.: Protein-bound iodine as an aid in the diagnosis of thyroid disease, *Canad. M. A. J.* **60**: 602–606 (June) 1949.
19. GREER, M. A. J.: The effect on endogenous thyroid activity of feeding desiccated thyroid to normal human subjects, *New England J. Med.* **244**: 385 (March 15) 1951.
20. DANOWSKI, T. S.; HUFF, S. J.; TARAIL, R.; WIRTH, P.; PETERS, J. H.; MATEER,

F. M., and GARVER, K.: Serum protein-bound iodine during ingestion of desiccated thyroid, *J. Clin. Endocrinol. & Metab.* **12**: 1572–1577 (Dec.) 1952.
21. ALBERT, A.: Thyroid gland, *Annual Rev. Physiol.* **14**: 481–498, 1952.
22. HICKEY, F. C., and BROWNELL, G. L.: Dynamic analysis of iodine metabolism in 4 normal subjects, *J. Clin. Endocrinol. & Metab.* **14**: 1423–1436 (Nov.) 1954.
23. BERSON, S. A., and YALOW, R. S.: Quantitative aspects of iodine metabolism. The exchangeable organic iodine pool, and the rates of thyroidal secretion, peripheral degradation and fecal excretion of endogenously synthesized organically bound iodine, *J. Clin. Invest.* **33**: 1533–1552 (Nov.) 1954.
24. STANBURY, J. B., and BROWNELL, G. L.: Multi-compartmented biologic models, *J. Clin. Endocrinol. & Metab.* **14**: 1079–1085 (Sept.) 1954.
25. DANOWSKI, T. S.; HUFF, S. J.; ERHARD, L. H.; PRICE, M.; BROWN, M.; WIRTH, P., and STEVENSON, S. S.: Protein-bound iodine levels in normal and in diabetic children, *A.M.A. Am. J. Dis. Child.* **84**: 5–10 (July) 1952.
26. PETERS, J. P., and MAN, E. B.: The relation of albumin to precipitable iodine of serum, *J. Clin. Invest.* **27**: 397–405 (July) 1948.
27. FAWCETT, D. M., and KIRKWOOD, S.: Role of the salivary glands in extrathyroidal iodine metabolism, *Science* **120**: 547–548 (Oct.) 1954.
28. THODE, H. G.; JAIMET, C. H., and KIRKWOOD, S.: Studies and diagnostic tests of salivary-gland and thyroid-gland function with radioiodine, *New England J. Med.* **251**: 129–134 (July 22) 1954.
29. ALBRIGHT, E. C.; LARSON, F. C., and DEISS, W. P.: Hypertrophy of salivary glands during treatment of myxedema with triiodothyronine, *J. Lab. & Clin. Med.* **44**: 762 (Nov.) 1954.
30. VAN MIDDLESWORTH, L., and BERRY, M. M.: Thyroid depression by stress, *Fed. Proc.* **10**: 140 (March) 1951.
31. O'NEAL, L. W., and HEINBECKER, P.: The response of the plasma protein-bound iodine of hypophysectomized dogs to injected thyrotropin: the influence of cortisone, *Endocrinology* **53**: 60–72 (July) 1953.
32. O'NEAL, L. W.: Plasma protein-bound iodine after intravenous injection of thyroxine in thyroidectomized dogs, *Endocrinology* **53**: 358–366 (Oct.) 1953.
33. ENGSTROM, W. W., and MARKARDT, B.: The effect of stress and cortisone on the circulating thyroid hormone, *J. Lab. & Clin. Med.* **44**: 793–794 (Nov.) 1954.
34. BONDY, P. K., and HAGEWOOD, M. A.: Effect of stress and cortisone on plasma protein-bound iodine and thyroxine metabolism in rats, *Proc. Soc. Exper. Biol. & Med.* **81**: 328–331 (Oct.) 1952.

INFLUENCE OF HORMONE THERAPY ON BODY FLUIDS, ELECTROLYTE BALANCE AND MUCOPOLYSACCHARIDES IN MYXEDEMA*†

ROSLYN WIENER, M.D.,** ANGELO IANNACCONE, M.D.,*** JULIUS EISENBERG, M.D.,‡ SOLOMON I. GRIBOFF, M.D.,§ ARTHUR W. LUDWIG, M.D. AND LOUIS J. SOFFER, M.D.

The Endocrine Research Laboratory and Clinic of the Department of Medicine, and the Department of Chemistry, The Mount Sinai Hospital, New York, New York

INTRODUCTION

MYXEDEMA is characterized by the extracellular deposit of large quantities of myxomatous material. This substance is present in both primary and pituitary myxedema (2), as well as in the circumscribed myxedema associated with thyrotoxicosis (3). By virtue of its rich content of acid-mucopolysaccharide (3), it would appear to be abnormally expanded ground substance, and hence to exert the water and electrolyte binding capacity of the latter (4). Thus it would be expected to play an important role in the fluid economy of the myxedematous individual. An early study by Byrom (5) documented a saline diuresis following the administration of desiccated thyroid to 2 myxedematous subjects. The assumption was made that the fluid and sodium were derived from the extracellular compartment.

It was our feeling that a serial study of body fluid compartments and electrolyte balances, in conjunction with serum and urinary mucopolysaccharides might provide some insight into the readjustment made in the myxedematous individual in response to specific therapy. Observations were also made during the administration of cortisone, an agent known to influence body fluid distribution (6), and known to exert an effect on connective tissue mucopolysaccharide (7).

Received for publication December 22, 1954.

* Aided by a grant from the United States Public Health Service.

† A preliminary report has been published elsewhere (1).

** Research Assistant in Medicine.

*** Dazian Foundation Fellow in Endocrinology. On leave of absence from the Institute of Medical Semeiology, University of Naples, Italy.

‡ Menke Fellow in Endocrinology.

§ Schenley Fellow in Endocrinology.

CLINICAL MATERIAL AND METHODS

This report is concerned with studies of 2 female patients with myxedema. The disease was of primary thyroidal origin in one, and secondary to pituitary insufficiency in the other. The experimental program for the patient with pituitary myxedema (R.H.) began with a period of thyrotropin administration. She was subsequently given desiccated thyroid, and finally cortisone concomitantly with desiccated thyroid. The patient with primary myxedema (D.G.) was initially given cortisone prior to the administration of desiccated thyroid alone. During the final period cortisone was again administered together with desiccated thyroid.

The patients were started on a constant, low-sodium (500 mg.; 22 mEq.) diet, adequate in protein and calories. Fluid intake was kept constant at 600 ml. per day. The calculated amount of sodium given in the sodium thiosulfate infusion for the determination of extracellular fluid space was added to the intake on the days when the determinations were made. Daily balances for sodium and chloride were estimated as the differences between calculated intakes and measured urinary excretions.

Extracellular fluid space and total body water were estimated from the volume distribution of thiosulfate (8) and of antipyrine (9), respectively. Determinations of sodium were made by a Perkin-Elmer internally standardized flame photometer, and of chloride by the Volhard method. Serum and urinary hexosamine were assayed by the method of Elson and Morgan (10) as modified by Boas (11), and serum mucoprotein by the method of Simkin *et al.* (12). Urinary neutral 17-ketosteroids were measured by the method of Holtorff and Koch (13), and formaldehydogenic steroids by the method of Corcoran and Page (14). Serum cholesterol was determined by the method of Schoenheimer and Sperry (15), phospholipids by the method of Sperry (16), and total lipids by the method of Bloor (17).

RESULTS

Effects of thyrotropin in the patient with pituitary myxedema

An increase in the thiosulfate and antipyrine spaces was noted after the first four daily injections of 25 mg. of thyrotropin (Armour), with a subsequent decrease in thiosulfate space below control values (see Table 1). The sodium and chloride balances, strongly negative during the control period, became positive under thyrotropin administration, but not sufficiently so to account for the early fluid gain. A rise in the serum sodium level was observed at the time of the increase in the extracellular fluid space, with a subsequent decline. No change from control values was observable in serum or urinary hexosamine, or in serum mucoprotein levels.

The thyrotropin period had to be terminated because of the development of water intoxication, characterized by nausea, vomiting, weakness and a slight drop in the blood pressure. This occurred during the performance of a water-loading test, and was probably an indication of adrenal insufficiency, as indicated by the decline in urinary formaldehydogenic steroids and by a serum sodium concentration of 122 mEq. per liter. Appropriate

therapy was instituted, with prompt recovery, and the patient was given 3 Gm. (51 mEq.) of oral sodium chloride supplement for the remainder of the study.

Effects of desiccated thyroid in the patient with primary, and in the patient with pituitary myxedema

During the period of desiccated thyroid administration, both patients demonstrated a transient expansion of the extracellular fluid space, with roughly parallel changes in total body water. There was an associated weight loss and an increase in urine volume in both subjects (see Tables 1 and 2).

a. Primary myxedema. It should be pointed out that the course of the patient with primary myxedema was complicated by an episode of incipient congestive failure at the beginning of the thyroid period. Appropriate measures were instituted, with an immediate large outpouring of sodium and chloride, weight loss, and a considerable drop in the venous pressure which had been at the upper limits of normal. The subsequent expansion of body fluid spaces, from the seventh to the twenty-ninth day of desiccated thyroid administration (experimental days 42–64) occurred in association with weight loss, continued negative sodium and chloride balances and venous pressure readings well within the range of normal. In view of these facts, a subsequent episode of congestive failure can be reasonably ruled out as an explanation of the fluid expansion observed later in the thyroid period.

This patient demonstrated an increase in urinary hexosamine output, with the maximal increase immediately preceding the maximal expansion of body fluid compartments. Serum mucoprotein was increased on one occasion without observable change in the concentration of serum hexosamine. The urinary formaldehydogenic steroids were greatly increased on two occasions prior to the greatest expansion of fluid spaces, and subsequently fell to control levels.

b. Pituitary myxedema. In the patient with pituitary myxedema, the overall sodium and chloride balances during the thyroid period were positive, but to a lesser degree than during the control period. There was an increase in urinary and serum hexosamine levels at the end of the thyroid period. Urinary formaldehydogenic steroids were absent at the end of the period.

Comparison of the body fluid changes during the thyrotropin period with those occurring during administration of desiccated thyroid reveals a similarity of pattern, with a more pronounced expansion of the extracellular fluid space, and one of longer duration, during the thyroid period.

TABLE 1. *R.H.* PITUITARY MYXEDEMA

Day of study	Period	Thiosulfate space L.	Thiosulfate space % body wt.	Antipyrine space L.	Antipyrine space % Body wt.	Body weight (Kg.)	Serum Na (mEq./L)	Serum K (mEq./L)	Serum mucoprotein (mg./100 ml.)	Serum hexosamine (mg./100 ml.)	Urine 17-KS (mg./d)	Urine FS* (mg./d)	Serum lipids: Cholesterol (mg./100 ml.)	Serum lipids: Phospholipids (mg./100 ml.)	Serum lipids: Total lipids (mg./100 ml.)	Days of study	Avg. daily Na bal. (mEq.)	Avg. daily Cl bal. (mEq.)	Avg. daily urine hexosamine (mg.)	Avg. daily urine vol. (ml.)
1	Control																			
3	↓	9.2	16.1			57.3	127	5.0	63	82	1.9	0.8								
5	Thyrotropin, 25 mg., after space determinations	9.1	15.9	20.8	36.3	57.3							372	322	1500	1–4	−34	−30	32	480
6	Thyrotropin, 25 mg.																			
7	Thyrotropin, 25 mg.																			
8	Thyrotropin, 25 mg.																			
9		10.3	18.2	23.0	41.7	55.1	138	4.6	62		1.2	0.1								
10	Thyrotropin, 25 mg.															5–22	+7	+9	33	480
12	Thyrotropin, 25 mg.																			
14		7.8	14.1	23.7	42.9	55.2	130	4.8	59	89	3.8	0.1	332	314	1410					
16	Thyrotropin, 25 mg.																			
18	Thyrotropin, 25 mg.																			
20	Thyrotropin, 25 mg.																			
22	Thyrotropin, 25 mg.																			
23	Water intoxication 75 mg. cortisone 20 ml. Lipo-adrenal ext. 4 Gm. NaCl						122	4.5												
24	3 Gm. NaCl supplement for rest of study						137	3.5												
26	Second control period																			
30	↓	7.8	13.8	22.9	40.5	56.5	136	4.6	60	76						26–30	+40	+14	47	380
31	Thyroid, 15 mg./day, started																			
36	↓ Thyroid, 30 mg./day	8.8	15.5	24.4	43.0	56.6	135	4.4	63	87	1.7	0.8				31–35	+14	+8	42	580
44	↓ Thyroid, 45 mg./day	12.1	21.6	24.5	43.8	55.9	138	4.4	63	78			281	305	1185	36–43	+23	+16	46	720
53	↓ Thyroid, 60 mg./day	10.8	19.8	24.4	44.6	54.5	136	4.4	63	83						44–52	+18	+12	44	620
63	↓ Cortisone, 150 mg./d, started after space determinations	9.4	17.6	21.7	40.7	53.3	142	4.5		95	1.5	0	176	230	870	53–62	+3	−5	54	580
67	↓	11.6	22.1	21.4	40.6	52.7	145	4.0	77	102			242	260	650	63–71	+9	−8	87	710
71	↓	8.6	16.1	21.3	39.9	53.4	139	3.9	69	98			234	276	740		(incomplete urine collections)			

*Formaldehydogenic steroids.

TABLE 2. *D.G.* PRIMARY MYXEDEMA

Day of study	Period	Thiosulfate space L.	Thiosulfate space % body wt.	Antipyrine space L.	Antipyrine space % Body wt.	Body weight (Kg.)	Serum Na (mEq./L)	Serum K (mEq./L)	Serum mucoprotein (mg./100 ml.)	Serum hexosamine (mg./100 ml.)	Urine 17-KS (mg./d)	Urine FS* (mg./d)	Serum lipids Cholesterol (mg./100 ml.)	Serum lipids Phospholipids (mg./100 ml.)	Serum lipids Total lipids (mg./100 ml.)	Days of study	Avg. daily Na bal. (mEq.)	Avg. daily Cl bal. (mEq.)	Avg. daily urine hexosamine (mg.)	Avg. daily urine vol. (ml.)
1–6	Control																			
7		13.0	15.5	34.8	41.5	84.0	133	4.8	61	84	2.5	0.7				1– 7	−12	−30	55	830
9	Cortisone, 150 mg./d., started after space determinations	13.6	16.1	36.2	42.8	84.5	131	3.8	50	93	2.8	0.5	288	236	800					
12		12.6	14.9	35.4	41.7	84.9	139	4.0												
14		13.7	16.2	36.0	42.5	84.8	137	4.0	70	92			288	272	980					
16		14.7	17.2	37.0	43.1	85.8	139	4.0		91	6.4	0.6	323	312	970	9–22	+29	+2	55	890
19		15.7	18.0	36.5	41.8	87.4	135	4.1		96										
21		15.0	17.4	37.1	42.9	86.5	136	4.2		96	9.3		297							
22	Last day of cortisone																			
23	Second control period	15.4	17.7	36.6	42.1	86.8	138	4.2	75	90			281	288	1030					
29		14.7	16.6	35.6	40.1	88.8	138	4.8	86	92	3.6	1.0	252	288	840	23–35	+17	+14	56	440
36	Thyroid, 30 mg./d., started after space determinations	15.6	17.4	36.1	40.1	89.9	129	5.2	86		5.6	1.4	286	382	900					
37						90.0														
38	†					90.7														
40	† Digoxin started					88.6										36–41	−47	−54	58	890
42		13.4	15.3	36.8	42.1	87.3			92	96			274	300	840					
45	†Thyroid, 60 mg./d.					85.7										42–49	−2	−3	53	1140
50		16.0	19.4	33.0	40.1	82.3			94			4.9	264	382		50–56	−22	+7	67	1080
57	Thyroid, 90 mg./d.	16.8	21.0	35.0	43.8	80.0	141	5.2	105		4.8	3.7	221	251	780	57–63	−37	−9	77	1070
64		17.9	22.9	37.4	47.7	78.4	136		83	94						64–68	−22	−18	62	1370
69	Thyroid, 120 mg./d.	15.6	20.3	31.7	41.1	77.0	138	5.2		93	5.1	0.6				69–76	−9	−3	66	830
77		13.9	18.3	33.0	43.4	76.0	144	5.1	80	87						77–83	−8	−5	66	820
84	Thyroid, 150 mg./d.	14.4	19.1	29.3	38.9	75.3			83	90	3.7	1.4	171	210	780	84–89	−26	−22	66	1040
89		13.2	17.5	33.2	44.0	75.5	144	5.2	86	90										
90	Cortisone, 150 mg./d., started																			
93		15.6	20.7	29.4	39.1	75.1	142	4.6	77	111										
97		16.9	22.6	31.2	41.6	75.0			93	87						90–107	−16	−14	81	830
100		15.5	20.7	33.0	44.1	74.8	141	5.1	90	91			152	223	805					
104		17.7	23.5	28.5	37.8	75.3	138	5.0	97											
107		16.0	21.3			75.0			91	90	3.7	3.8	171	230	760					

* Formaldehydrogenic steroids.
† Mercuhydrin, 2 cc. i.m.

Response to cortisone

The patient with primary myxedema, when given cortisone while still in the myxedematous state, demonstrated a slow, moderate expansion of the extracellular fluid space, associated with sodium retention, a rise in serum sodium concentration, and a gain in weight. Some rise was noted in the serum mucoprotein level, but no change in the serum or urinary hexosamine values. During the joint administration of cortisone and desiccated thyroid, after fifty-four days on thyroid alone, there was prompt and sustained expansion of the extracellular fluid space. Sodium and chloride balances remained negative, as they had been during the immediately preceding thyroid period, and body weight remained constant. Urinary hexosamine values were sharply increased over those obtained previously, and there was a rise in serum mucoprotein and hexosamine values.

During the period of thyroid-cortisone administration, the patient with pituitary myxedema showed considerable transient expansion of the extracellular fluid space, without a corresponding change in total body water or body weight. The balance studies indicated an overall slightly positive sodium, and a slightly negative chloride balance. As observed in the other patient, there was a pronounced rise in urinary hexosamine. Slight rises were also noted in the serum mucopolysaccharide fractions.

Serum lipids

The serum lipid alterations during the course of this study are of some interest. In the patient with primary myxedema, initial values for all lipid fractions measured (cholesterol, phospholipids and total lipids) were at the upper limits of normal. An elevation of all fractions occurred during the initial cortisone period, as has been observed in normal subjects after cortisone administration (18). There was the expected progressive fall in lipid fractions during treatment with desiccated thyroid, without significant alteration during thyroid-cortisone administration, in contrast to the rise observed with cortisone alone.

The patient with pituitary myxedema showed an initial gross hyperlipemia, with a slight reduction in the serum cholesterol level during thyrotropin administration. There was a substantial reduction of all serum lipid fractions during thyroid treatment, with a subsequent moderate elevation of serum cholesterol and phospholipid concentrations after the addition of cortisone.

DISCUSSION

We suggest the possibility that, in both patients during the administration of desiccated thyroid, and in the patient with pituitary myxedema

during the administration of thyrotropin, the observed expansion of the body fluid spaces was a consequence of the breakdown of myxedematous material. This hypothesis would account for the fluid expansion as a result of liberation from the myxomatous material of previously bound fluid and electrolyte into the measurable thiosulfate and antipyrine spaces. The increased urinary excretion of sodium and chloride, as well as the increase in urine volume, is consistent with this hypothesis. That the expansion of the body fluid spaces is an endogenous mechanism is indicated by the weight loss in both patients, as well as by the lack of correspondence between the sequential space measurements, and the direction or magnitude of the electrolyte balances.

In an effort to observe changes occurring in the myxedematous material during treatment, we studied the serum and urinary mucopolysaccharides, with serum fractions measured both as "hexosamine" and as "mucoprotein," and the urinary fraction as "hexosamine." The study indicated a modest increase of urinary hexosamine in both patients during desiccated thyroid administration. The changes in serum fractions were less striking. However, a slight rise in the serum hexosamine level was noted in the patient with pituitary myxedema, and in the patient with primary myxedema there occurred a transient rise in serum mucoprotein level which immediately preceded the maximal increase in body fluid spaces. Though the magnitude of increase in mucopolysaccharide fractions was not great, and was not uniform in all fractions measured, correlation was indicated between the increase in body fluid spaces and the rise in measurable serum and urinary mucopolysaccharide concentrations.

The patient with primary myxedema demonstrated a considerable increase in the urinary formaldehydogenic steroids immediately preceding the greatest fluid increase, suggesting an adrenal factor in the production of the fluid phase increase. It is also possible that the increase in all urinary excretion products, as well as the increase in urinary volume, was a consequence of the enhanced renal function occurring as a result of thyroid treatment in myxedema, as has been repeatedly demonstrated (19, 20, 21).

The thyroid-cortisone period in both patients was characterized by a considerable increase in urinary hexosamine output, in association with extracellular fluid space expansion. Such an increase did not occur in the patient with primary myxedema receiving cortisone alone. This might suggest the potentiating effect of cortisone on residual myxedematous material, or on normal connective-tissue ground substance. This interpretation might be substantiated by Asboe-Hansen's (22) observation of a decrease in metachromatic staining connective-tissue mucopolysaccharide after the administration of ACTH.

CASE SUMMARIES

Patient 1. R.H., MSH 19616. A 33-year-old white female was admitted with complaints of marked weakness, fatigue, slowness of speech and of thought processes, weight gain, and puffiness of the face, hands and feet of one year's duration. The skin had been thickened and dry, and body hair had been sparse for several years. Breast development had been normal; menarche had occurred at age 13, but menses had ceased spontaneously at age 23, ten years prior to admission. There had been no pregnancy in four years of marriage. The physical examination revealed a short, somewhat obese female, with non-pitting edema of the face, hands, and feet. Cerebration and speech were slow, and the voice was harsh. The skin was sallow, dry and thickened. The visual fields and the fundi were normal. The thyroid gland was not palpable. The breasts were large and fatty. The pulse rate was 68 per minute, and blood pressure 100/80 mm.Hg. The axillary, pubic and eyebrow hair were sparse, and the scalp hair thick and dry. The tendon reflexes showed a slow relaxation phase. Laboratory studies showed a mild hypochromic, normocytic anemia, with normal white blood cell and differential counts. Urinary findings were normal. Values for fasting blood sugar and glucose tolerance were normal, as were those for sedimentation rate, blood Kahn, and blood urea nitrogen. Serum protein-bound iodine concentration was 2.6 gamma per 100 ml., and the basal metabolic rate was −41 per cent. After three daily injections of thyrotropin, there occurred an increase in the thyroidal uptake of a tracer dose of I^{131} from 8 per cent to 36 per cent of the administered dose, and an increase in the level of serum PBI^{131}. There was a normal decrease in the eosinophil count after the injection of corticotropin. Roentgen examination of the chest showed globular enlargement of the heart; the skull plate was normal.

Patient 2. D.G., MSH 16309. A 64-year-old white female was admitted with complaints of weakness, lethargy, cold sensitivity, coarse dry skin, weight gain, and intermittent dyspnea and orthopnea of eight years' duration. There had been two episodes of pain and swelling in the region of the thyroid gland, twelve and nine years prior to admission. Desiccated thyroid had been administered intermittently over a period of two years, from eight to six years before admission, with relief of symptoms. However, the complaints had recurred after omission of medication. She had not been treated with thyroid for six years prior to this study. The physical examination revealed a short, obese female with slow, thick, hoarse speech and non-pitting edema of the face, hands and torso. The skin was pale, dry and coarse, and the tongue was enlarged. The thyroid gland was not palpable. The pulse rate was 54 per minute and the blood pressure was 110/60 mm. Hg. All reflexes were sluggish, with a prolonged relaxation phase. Laboratory studies showed a moderate hypochromic, microcytic anemia, with normal white blood cell and differential counts. Urinary findings were normal. Levels of blood urea nitrogen, fasting blood sugar, and serum proteins were normal. The roentgenogram of the skull was normal; that of the chest revealed an increased transverse diameter of the heart. The electrocardiogram showed sinus bradycardia, low voltage in all leads, and inverted T waves in leads I, II, AVF, and V5. The thyroidal uptake of a tracer dose of I^{131} was 11 per cent in twenty-four hours, and the basal metabolic rate was −26 per cent. There was a normal decrease in the eosinophil count after the injection of corticotropin.

SUMMARY

Two female patients, 1 with primary, and 1 with pituitary myxedema were studied.

The patient with pituitary myxedema, during thyrotropin treatment, showed expansion of extracellular fluid space (thiosulfate) and total body water (antipyrine), with subsequent contraction of extracellular fluid space. There was an associated slight retention of sodium and chloride, and a slight weight loss.

During treatment with desiccated thyroid, both patients demonstrated transient expansion of extracellular fluid space with essentially parallel changes in total body water, weight loss, and increased urine volume. Sodium and chloride balances became negative in the patient with primary myxedema. The other patient showed sodium and chloride retention, but to a lesser degree than during the preceding control period. In the patient with primary myxedema there was an increase in urinary hexosamine and a transient increase in the level of serum mucoprotein; in the other patient, there occurred an increase in urinary and serum hexosamine levels at the end of the period.

The patient with primary myxedema, under treatment with cortisone prior to thyroid administration, demonstrated moderate expansion of the extracellular fluid space, associated with sodium and chloride retention and weight gain. Some rise in the concentration of serum mucoprotein was noted. During combined thyroid-cortisone administration, there occurred greater expansion of extracellular fluid space, and negative sodium and chloride balances, with constant weight. The patient with pituitary myxedema, during treatment with thyroid and cortisone, showed transient extracellular space expansion. In both patients there was a considerable increase in urinary hexosamine during combined thyroid-cortisone treatment, not observable in the patient with primary myxedema receiving cortisone alone. There was a slight rise in serum mucopolysaccharide fractions in both patients during combined therapy.

REFERENCES

1. Soffer, L. J.; Iannaccone, A.; Wiener, R.; Griboff, S. I., and Eisenberg, J.: Body fluids and electrolyte balance in myxedema, *Acta endocrinol.* **17**: 418, 1954.
2. Gabrilove, J. L.; Ludwig, A. W., and Soffer, L. J.: Effect of thyroid hormone and thyrotropin on the ground substance and connective tissue, (abstract) *J. Clin. Endocrinol. & Metab.* **12**: 966, 1952.
3. Watson, E. N., and Pearce, R. H.: The mucopolysaccharide content of the skin in localized (pretibial) myxedema. II., *Am. J. Clin. Path.* **19**: 442, 1949.
4. Ludwig, A. W.; Chen, D. K., and Soffer, L. J.: The relationship between connective tissue mucopolysaccharide content and tissue electrolyte composition, (abstract) *J. Clin. Endocrinol. & Metab.* **12**: 965, 1952.
5. Byrom, F. B.: The nature of myxoedema, *Clin. Sci.* **1**: 273, 1934.
6. Levitt, M. F., and Bader, M. E.: Effect of cortisone and ACTH on fluid and electrolyte distribution in man, *Am. J. Med.* **11**: 715, 1951.
7. Asboe-Hansen, G.: The variability in the hyaluronic acid content of the dermal

connective tissue under the influence of thyroid hormone, *Acta dermat.-venereol.* **30**: 221, 1950.

8. Cardozo, R. H., and Edelman, I. S.: The volume of distribution of sodium thiosulfate as a measure of the extracellular fluid space, *J. Clin. Invest.* **31**: 280, 1952.
9. Soberman, R.; Brodie, B. B.; Levy, B. B.; Axelrod, J.; Hollander, V., and Steele, J. M.: The use of antipyrine in the measurement of total body water in man, *J. Biol. Chem.* **179**: 31, 1949.
10. Elson, L. A., and Morgan, W. T. J.: A colorimetric method for the determination of glucosamine and chondrosamine, *Biochem. J.* **27**: 1824, 1933.
11. Boas, N. F.: Method for the determination of hexosamines in tissues, *J. Biol. Chem.* **204**: 553, 1953.
12. Simkin, B.; Bergman, H. C., and Prinzmetal, M.: Studies on coronary circulation, V. Quantitative changes in a serum mucoprotein following the occurrence of myocardial infarction, *Am. J. Med.* **6**: 734, 1949.
13. Holtorff, A. F., and Koch, F. C.: The colorimetric estimation of 17-ketosteroids and their application to urine extracts, *J. Biol. Chem.* **135**: 777, 1940.
14. Corcoran, A. C., and Page, I. H.: Methods for the chemical determination of corticosteroids in urine and plasma, *J. Lab. & Clin. Med.* **33**: 1326, 1948.
15. Schoenheimer, R., and Sperry, W. M.: A micromethod for the determination of free and combined cholesterol, *J. Biol. Chem.* **106**: 745, 1934.
16. Sperry, W. M.: Electrophotometric microdetermination of phosphorus in lipid extracts, *Indust. & Engin. Chem.*, Anal. ed. **14**: 88, 1942.
17. Bloor, W. R.: A method for the determination of fat in small amounts of blood, *J. Biol. Chem.* **17**: 377, 1914.
18. Adlersberg, D.; Schaefer, L. E., and Drachman, S. R.: Studies on hormonal control of serum lipid partition in man, *J. Clin. Endocrinol.* **11**: 67, 1951.
19. Corcoran, A. C., and Page, I. H.: Specific renal functions in hyperthyroidism and myxedema. Effects of treatment, *J. Clin. Endocrinol.* **7**: 801, 1947.
20. Luft, R., and Sjögren, B.: The significance of the adenohypophysis, adrenal cortex and thyroid in renal function in man, *Acta endocrinol.* **4**: 351, 1950.
21. Davies, C. E.; MacKinnon, J., and Platta, M. M.: Renal circulation and cardiac output in "low-output" heart failure and in myxoedema, *Brit. M. J.* **2**: 595, 1952.
22. Asboe-Hansen, G.: Effect of the adrenocorticotropic hormone of the pituitary on mesenchymal tissue, *Scandinav. J. Clin. & Lab. Invest.* **2**: 271, 1950.

JUVENILE HYPERPARATHYROIDISM IN ASSOCIATION WITH PEPTIC ULCER

HIMEO TSUMORI, M.D., ESTHER JENSEN, M.D., ARTHUR J. HUNNICUTT, M.D., NADINE FOREMAN, M.D. AND LAURANCE W. KINSELL, M.D.

The Departments of Pediatrics, Radiology, and Surgery, and the Institute for Metabolic Research of the Highland Alameda County Hospital, Oakland, California

HYPERPARATHYROIDISM in childhood is a relatively rare occurrence (1, 2). The present report is of a 10-year-old child in whom the disease was associated with peptic ulcer. The diagnosis was made difficult by a traumatic fracture of the femur with consequent immobilization.

CASE REPORT

Patient WAD, the seventh child of a 37-year-old mother, was considered to be in excellent health until 1953, when he manifested fatigue and abdominal distress. As the result of the latter, in August 1954, an appendectomy was performed on the basis of a preoperative diagnosis of acute appendicitis. The pathologic report of the surgical specimen was "possible tuberculoma of the appendix." Results of tuberculin and histoplasmin skin tests were negative. X-ray examination showed a normal chest. The symptoms subsided following operation, and the patient was discharged to the clinic.

Because of recurrence of abdominal distress, intravenous pyelograms and upper gastro-intestinal x-ray series were carried out in October 1953. The pyelogram showed a small dense area in the right kidney region, as well as coarsening of the trabecular pattern of the pelvic bone. The gastro-intestinal series showed an active duodenal ulcer, with associated duodenal deformity. Repetition of the series in December showed deformity but no crater. X-ray examination showed the gallbladder to be normal. The patient received "usual ulcer therapy" with improvement, and was not seen from January until June, 1954. On June 21, he sustained a fracture of the right femur as the result of an automobile accident. X-ray examination of the pelvis showed increased coarsening of the trabecular markings. The bone in the region of the fracture was not remarkable. Dental films revealed essentially normal lamina dura.

Laboratory examinations (performed in the clinical laboratory) showed a serum calcium level of 15.9 mg. per 100 ml. and alkaline phosphatase, 13.0 King-Armstrong units. There was a 4+ precipitate with the Sulkowitch test for urinary calcium. Phenolsulfonphthalein excretion was 50 per cent in one hour. Blood creatinine concentration was reported as 3.3 mg. per 100 ml.

Two days following the fracture, while in traction, the patient had increasing epigastric pain. Ingestion of food resulted in nausea and vomiting. Antacids and antispasmodics were not retained.

On June 28, the serum calcium level was 15.6 mg. per 100 ml., inorganic phosphorus

Received for publication December 27, 1954.

4.26 mg., and alkaline phosphatase 8.15 Bodansky units (research laboratory). The serum albumin level was 3.94 Gm. and the globulin, 2.41 Gm. per 100 ml.

In view of the history and findings, the provisional diagnoses included:

1. Hypercalcemia on the basis of immobilization superimposed upon fracture, in an actively growing child.
2. Hyperparathyroidism.
3. Recurrence of peptic ulcer.

The patient was started on a diet low in calcium and phosphorus and high in fluids. He also received testosterone for its anabolic effects.

On this program, the patient was clinically somewhat improved, but on July 9 the serum level of calcium was 16.3 mg. per 100 ml., phosphorus 2.88 mg., and alkaline phosphatase 7.62 Bodansky units. Two days later the serum calcium level was 17.3 mg. and phosphorus 2.7 mg. per 100 ml. In view of the serum values, the probability of a small renal calculus, and some abnormal skeletal findings prior to fracture, a diagnosis of hyperparathyroidism seemed inescapable.

An adenoma of the left lower parathyroid gland (lying adjacent to a normal left upper parathyroid) was removed on July 14. The diagnosis was confirmed histologically by Dr. Grace Hyde.

The usual precautions were taken to cope with possible postoperative hypocalcemia, but the postoperative course was completely uneventful. The chemical changes in the blood serum were as follows:

	Calcium (mg./100 ml.)	Phosphorus (mg./100 ml.)	Alk. phosphatase (Bodansky units)
July 15	12.5	3.47	
July 16	11.1	3.08	
July 19	12.5	3.70	3.7
July 21	9.3		
July 23	10.0	5.15	5.25

Healing of the fracture progressed normally.

Of particular interest was the change in the behavior pattern of the child. Prior to removal of the parathyroid adenoma, he tended to be irritable and morose. Within a week after surgery, and thereafter, he was free of all complaints and behaved like a thoroughly normal and happy 10-year-old child.

DISCUSSION

That a major degree of hypercalcemia can occur with immobilization in the growing child has been demonstrated by Albright (3). Such hypercalcemia should respond to vigorous hydration and should not be associated with hypophosphatemia. In the child reported here, the lack of response to high fluid intake, plus pre-existing urinary-tract calcification and minimal but definite pre-existing skeletal abnormality, led to a correct preoperative diagnosis.

The relationship between duodenal ulcer and hyperparathyroidism is statistically highly significant. The mechanism of the relationship is as yet unclear. Rogers *et al.* believe that treatment of peptic ulcer can result in parathyroid hyperfunction (4). Black (5) states that at the Mayo Clinic

24 per cent of all patients with proven hyperparathyroidism had a peptic ulcer, and an additional 15 to 20 per cent had a history strongly suggestive of peptic ulcer. Schiffrin (6) injected parathyroid extract into dogs and reported a definite change in volume, and an increase in acidity and pepsin content of the gastric secretions.

In view of the rarity of peptic ulcer in children, it may be well to carry out at least one serum calcium and phosphorus determination in all juvenile patients who present a proven or suspected ulcer syndrome.

REFERENCES

1. ANSPACH, W. E., and CLIFTON, W. M.: Hyperparathyroidism in children; report of two cases, *Am. J. Dis. Child.* **58**: 540–557, 1939.
2. *a.* BALCH, H. E.; SPIEGEL, E. H.; UPTON, A. L., and KINSELL, L. W.: Teaching Clinic—Hyperparathyroidism: report of 2 cases with some relatively unusual manifestations, *J. Clin. Endocrinol. & Metab.* **13**: 733–738, 1953.
b. FROHNER, RICHARD N., and WOLGAMOT, JOHN C: Primary hyperparathyroidism: 5 cases in one family, *Ann. Int. Med.* **40**: 765–773, 1954.
c. NORRIS, E. H.: Collective review. Parathyroid adenoma; study of 322 cases, *Internat. Abstr. Surg.* **84**: 1–41, 1947 (*Surg., Gynec. & Obst.* Jan. 1947).
3. ALBRIGHT, F., and REIFENSTEIN, E. C.: Parathyroid Glands and Metabolic Bone Disease; Selected Studies. Baltimore, Williams & Wilkins Co., 1948.
4. ROGERS, H. M.; KEATING, F. R., JR.; MORLOCK, C. G., and BARKER, N. W.: Primary hypertrophy and hyperplasia of the parathyroid glands associated with duodenal ulcer; report of additional case with special reference to metabolic, gastrointestinal and vascular manifestations, *Arch. Int. Med.* **79**: 307–321, 1947.
5. BLACK, B. M.: Hyperparathyroidism. Springfield, Ill., Charles C Thomas, Publisher, 1953.
6. SCHIFFRIN, M. J.: Relationship between parathyroid and the gastric glands in the dog. *Am. J. Physiol.* **135**: 660–669, 1942.

Teaching Clinic

AN ADRENAL TUMOR, CLINICALLY DOUBTFUL; DIAGNOSIS ESTABLISHED BY EXTRA-PERITONEAL PNEUMOGRAPHY

THE primary purpose of this report is to stress, especially from a teaching standpoint, the invaluable aid to be derived from the introduction of oxygen presacrally to visualize an adrenal tumor. In the case described here, except for this procedure the tumor would almost certainly have gone undetected. It is hoped that a perusal of the following clinical narrative will convince the reader that there was in this patient relatively little evidence to support the diagnosis of Cushing's disease, let alone adrenal tumor, in contrast to many weighty negative findings against such a diagnosis.

CASE REPORT

In March 1955, a 40-year-old married woman was referred to the writer by Dr. Hyman J. Shinbane of Los Angeles. He suspected Cushing's disease; however, our questioning and physical examination, as well as the laboratory tests, led us to believe that such a diagnosis in her case was dubious.

Her principal complaint was suboccipital and bitemporal headaches, which she had had for many years, but which had been worse and occurred more frequently during the previous six months. They might last for several days or even a week, and they were not unilateral. They were not accompanied by diplopia or fortification spectrum, and rarely by vomiting, nor were they relieved or even mitigated by the recumbent posture. Roentgenograms of the skull revealed hyperostosis frontalis and parietalis, but at this juncture the headaches could not be related to this finding, which is often asymptomatic.

The patient complained also of listlessness and fatigue, which were not at all characteristic of her former energetic habits. She had become somewhat drowsy in the daytime, and her usual zest and eager activity had diminished noticeably. She volunteered (and this was corroborated by her husband and concurred in by Dr. Shinbane, who had attended her for many years) that her personality had undergone a change in the direction of irritability, whereas formerly she had always had a placid, pleasant disposition. This factor was not evident to us; she was truly most agreeable, cheerful and cooperative, but in any case such changes at her age could easily be assigned to an approaching menopause.

A third complaint was stubborn obesity; she had always been heavy and found it difficult to lose weight.

The foregoing, and only, symptoms were hardly of a nature to suggest a diagnosis of Cushing's disease. Furthermore, the anamnesis revealed that whereas she had had

several episodes of amenorrhea in the past, her menstrual periods had been fairly regular during the previous two years. This story was actually out of line with progressive Cushing's disease. She was the mother of two healthy sons.

On physical examination there was typical truncal and moon-faced obesity as first described by Cushing, sparing the shapely arms and legs. However, in going over her old photographs, it was difficult to say that there had been any great dramatic change (Fig. 1). She had been obese for many years, the weight increasing gradually. Moreover, she lacked kyphosis and buffalo hump.

She had thick rather than thin skin, she had none of the purplish striae of Cushing's disease, and she was able to run up and down stairs without any muscular weakness. Moreover, during the time she was in the hospital many venipunctures were necessary for laboratory studies, and later for intravenous therapy, but at no time did she exhibit the ecchymoses and bruisability so commonly noted in classic Cushing's syndrome.

The degree of hirsutism was mild and confined to the face; there was no heterosexual hypertrichosis elsewhere on the body. The clitoris was not enlarged.

FIGURE 1

Her face was slightly flushed but could hardly be called plethoric (Volhard's "roter Hochdurck"). Though her blood pressure had been slightly elevated (155 to 160 systolic and 95 to 100 diastolic) prior to her admission to the University of California Hospital, it remained at normal levels throughout her three weeks' stay.

The physical examination, therefore, like the symptomatology, failed to be impressive in respect to a diagnosis of hyperadrenocorticism.

In view of the paucity of positive signs and symptoms favoring Cushing's disorder, it was somewhat surprising to note in the preliminary report of Dr. Shinbane that the urinary 17-ketosteroid excretion in twenty-four hours was 28.8 mg., a finding which was confirmed in the laboratory of Dr. Peter H. Forsham in San Francisco (normal for adult females, 10 mg. $\pm$ 5 mg.). The excretion of 17-hydroxycorticosteroids, which had been reported by Dr. Shinbane as 26.8 mg. of E per twenty-four hours (method of Reddy, Jenkins and Thorn (1)), although only 15 mg. of F per twenty-four hours when repeated here (method of DiRaimondo *et al.* (2)), was still above the normal range of 7 mg. $\pm$ 4 mg of F per twenty-four hours for women. These results suggested definite adrenocortical dysfunction in spite of the scarcity of clinical signs and symptoms. As emphasized by Laidlaw *et al.* (3) and others, an adenoma is usually accompanied by a small but definite increase in 17-hydroxycorticoid excretion.

Although the hematocrit and hemoglobin levels were at the upper limits of normal, they were by no means consistent with a diagnosis of polycythemia, and both lymphopenia and eosinopenia were lacking.

A search for osteoporosis excluded this Cushing complication, nor were there any rib or vertebral fractures. However, on the positive side of laboratory findings were mild glycosuria and a definitely impaired carbohydrate tolerance as manifested by a diabetic type of glucose tolerance curve following 100 Gm. of glucose given orally: fasting blood sugar, 146 mg. per 100 ml.; 8:30 A.M., 224 mg.; 9:00 A.M., 278 mg.; and 10:00 A.M., 230 mg. per 100 ml. (method of Somogyi).

The referring physician sent along an x-ray film of the skull (lateral view), and this disclosed an unusually circular, globular sella turcica, which was considered to be at the upper limit of normal in size, and perhaps even slightly enlarged. Its rounded shape suggested ballooning from within, but a basophil adenoma sufficiently large to expand the sella in this fashion is most unusual.

It is altogether likely that we would not have proceeded further had not the patient come from quite a distance. There was also the written suspicion of her referring physician that she might be suffering from Cushing's disease. Therefore, before dismissing her, it was decided to subject her to intravenous urography and retroperitoneal pneumography.[1]

---

[1] Only rarely, and then unfortunately too late, is an adrenal tumor large enough to be palpated, even in patients under anesthesia. Such tumors are too high up under the diaphragm. Some are revealed by intravenous urography, but only if they are fairly large. Displacement and flattening of the upper calyx of the kidney are more clearly demonstrated by retrograde pyelography—but again, only if the tumor is large enough to push the kidney downward, or displace or rotate it. Presacral pneumography not only demonstrates smaller tumors which otherwise would be missed but, when properly executed using oxygen and carefully controlled pressure, is no more unpleasant or risky than retrograde pyelography. Insufflation of air around the kidney by the method of Carelli is a truly hazardous procedure, and a by no means negligible mortality from air

The urologist, Dr. Frank Hinman, Jr., surmised that there was a left-sided adrenal tumor on the basis of the urograms alone, the left kidney being displaced slightly to the left and rotated just a bit anteriorly. However, the upper calyx was not flattened or depressed downward, and Dr. Gilbert S. Gordan and I did not consider the slender foregoing evidence

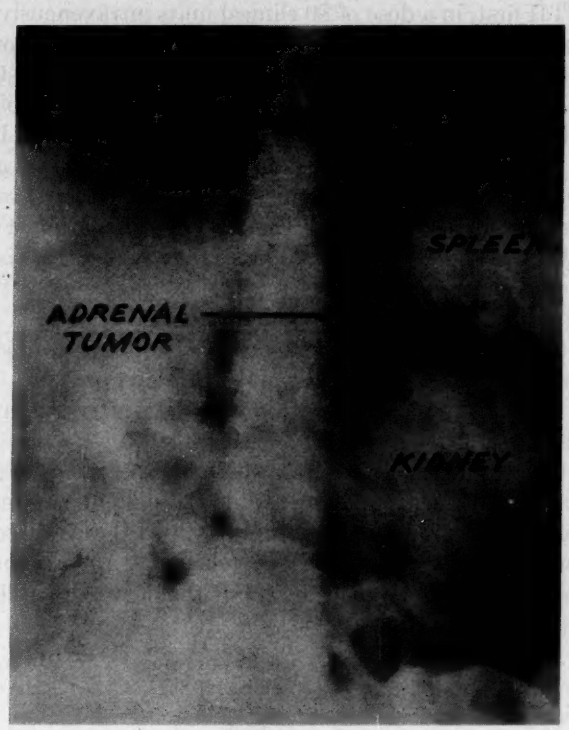

FIG. 2. Tomogram showing tumor of left adrenal.

sufficiently conclusive to warrant a left adrenal exploration. Indeed, we would have granted generous odds against the presence of an adrenal tumor.

On March 7, 1955, Dr. Hinman inserted 1,300 cc. of oxygen under a pressure of 30 cm. of water presacrally, and tomograms were obtained thereafter. To our great surprise these clearly demonstrated a tumor of the left adrenal, ovoid in shape, measuring 4 cm. transversely and 7 cm. in length (Fig. 2). The right adrenal was not well visualized, but on some of

emboli has resulted. This procedure is not to be confused with presacral pneumography, following which the only uncomfortable symptom is a feeling of tightness in the neck for about twelve to twenty-four hours.

the films a shadow could be discerned consistent with a somewhat atrophied right adrenal gland (4).

Operation being agreed upon, Dr. Gordan was placed in charge of the preoperative and postoperative medical management. The right adrenal gland was stimulated, to avoid postoperative hypocorticism. The patient received ACTH first, in a dose of 80 clinical units intravenously, on March 11. This caused a pronounced rise in the 17-ketosteroid and corticoid output; the former rose to 50 mg., and the latter to 66 mg. per twenty-four hours. ACTH gel was then given intramuscularly in a dose of 40 clinical units twice daily for the following three days. On the third day Dr. Hinman successfully removed the tumor together with the left adrenal gland which was plastered across the top of the tumor. The adrenal itself seemed to be hypertrophied, probably because of the ACTH stimulation. Fortunately, the tumor was completely encapsulated and subsequently, histologically, was found to be a benign adenoma.

Postoperatively, the patient responded remarkably well. It seemed apparent that the remaining right adrenal cortex had taken over successfully, as there was no change in the weight, pulse or blood pressure. The dose of ACTH was rapidly tapered, and within four days after the operation she was taking none.

It is of interest that the glycosuria, which had been inconstant prior to administration of ACTH, rose to 4+ while the patient was receiving this hormone, but disappeared almost entirely as ACTH was discontinued. A glucose tolerance test repeated on the tenth day after removal of the adrenal tumor showed less impairment, but the curve was still diabetic in type:

	6 Days preop. (mg./100 ml.)	10 Days postop. (mg./100 ml.)
Fasting blood sugar	146	116
1/2 hour	224	194
1 hour	278	228
2 hours	230	197
3 hours	—	145

The patient returned to her home ten days after removal of the tumor, in good health. There has been no further treatment. It is planned to check the glucose tolerance and to determine the 17-ketosteroid and corticoid outputs in about three months. If the patient has not lost weight and the mild diabetes persists, a reduction regimen will be instituted. Since removal of the adrenal tumor, headaches have ceased and the patient, her husband, Dr. Shinbane, and her friends have all noticed a return to her former, pleasant, placid disposition, with complete loss of the tense irritability which had characterized her temperament preoperatively.

In this case of adrenal tumor causing a sort of forme fruste or early stage of Cushing's disease in which anabolic and catabolic hormones were so well balanced as to leave many of the clinical end-points unaffected, the correct diagnosis was based on the abnormal urinary steroid values, but above all on the clear-cut demonstration of the tumor by retroperitoneal pneumography.

H. LISSER, M.D.

The Endocrine Clinic,
Department of Medicine,
University of California School of Medicine,
San Francisco, California

REFERENCES

1. REDDY, W. J.; JENKINS, D., and THORN, G. W.: Estimation of 17-hydroxycorticoids in urine, *Metabolism* **1**: 511, 1952.
2. DI RAIMONDO, V. C.; ORR, R. H.; ISLAND, D.; RINFRET, A. P., and FORSHAM, P. H.: An improved steroidogenic assay of ACTH in man, *Metabolism* **4**: 110, 1955.
3. LAIDLAW, J. C.; JENKINS, D.; REDDY, W. J.; HARRISON, J. H., and THORN, G. W.: Diagnosis of hyperadrenocorticism, (abstract) *J. Clin. Endocrinol. & Metab.* **14**: 781, 1954.
4. STEINBACH, H. L., and SMITH, D. R.: Extraperitoneal pneumography in diagnosis of retroperitoneal tumors, *Arch. Surg.* **70**: 161, 1955.

Letters to the Editor

EXCRETION OF 17-HYDROXYCORTICOSTEROIDS DURING THE MENSTRUAL CYCLE*

To the Editor:

In studying the urinary excretion of 17-hydroxycorticosteroids (17-OH-CS) by normal persons of both sexes, using the method of Porter and Silber (1) as modified by Glenn and Nelson (2), an occasional result higher than the reported range of normal values—2.3 to 5.8 mg. per twenty-four hours (3)—was found in females of premenopausal age. This prompted us to study the variation in 17-OH-CS output during the menstrual cycle. The results of these observations are presented in Table 1. Although none of the individuals subjected to this study showed an output of 17-OH-CS outside of the normal limits, nevertheless, the cyclical variation in the excretion pattern is of interest.

Sandberg *et al.* (3), using the method of Glenn and Nelson (2), studied the daily 17-OH-CS excretion of a 28-year-old normal female for fifteen days preceding the onset of menstruation. It can be calculated from their table that the average amount of 17-OH-CS excreted during the second week before menstruation is 15 per cent higher than the average amount excreted during the week immediately preceding menstruation.

In the present study we found that after β-glucuronidase hydrolysis the excretion of total 17-OH-CS (Porter-Silber reactive corticosteroids) by young females varied with a certain regularity during the menstrual cycle. Six normal young women, 20 to 28 years of age, were studied. Each subject collected refrigerated 24-hour urine specimens once weekly during her menstrual cycle; aliquots of the urine specimens were stored in a deep freeze. The 17-OH-CS levels were determined by the method of Glenn and Nelson (2), with slight modifications:

After adjusting the urine to pH 5.0 with 40 per cent sulfuric acid, 10-ml. aliquots were taken and 1.0 ml. each of acetic acid (0.2M), sodium acetate (0.2M), and liver β-glucuronidase (5000 Fishman units of ketodase obtained from Warner-Chilcott Co., Morristown, N. J.) (4) were added. The mixture (pH 4.8) was incubated for eighteen hours at 48°C. Instead of 4 per cent, 2 per cent methanol was used for washing the columns. Each determination was run in duplicate with and without the addition of 25

* This investigation was supported by a grant from the National Institutes of Health, Division of Neurological Diseases and Blindness.

TABLE 1. URINARY 17-HYDROXYCORTICOID EXCRETION OF YOUNG FEMALES DURING EACH WEEK OF THEIR MENSTRUAL CYCLE

	Weeks after the onset of the menstrual period (mg. of 17-OH-CS excreted in 24 hours.)			
	1st	*2nd*	*3rd*	*4th*
	2.39	5.18	5.73	—
	3.31	3.52	4.64	—
	3.93	2.94	5.16	3.83
	3.60	4.41	4.69	4.49
	4.96	3.83	3.67	3.64
	2.30	4.69	4.92	—
Mean	*3.42*	*4.09*	*4.80*	*3.20*
P(*)	$P_{1,2} < .01$	$P_{2,3} > .05$	$P_{3,4} < .05$	
	$P_{1,3} < .05$	$P_{2,4} > .05$		
	$P_{1,4} > .05$			

(*) P stands for the probability of results occurring by chance only, determined by statistical calculations, employing *t*-values of Student's *t*-Test [5]. The subscripts are used to indicate the weekly periods which are compared with each other.

μg. of cortisone alcohol (obtained from Sigma Chemical Co., St. Louis, Mo.) with duplicate sulfuric acid blanks. For color development 1.5 ml. of methanol and 2.25 ml. of phenylhydrazine-sulfuric acid reagent were used.

The 17-OH-CS excretion during the second and third weeks of the menstrual cycle was significantly higher than that during the first and fourth weeks (Table 1). The highest values were found during the third week in 5 out of 6 cases. It appears important, therefore, to correlate the period of the menstrual cycle with the 24-hour 17-OH-CS excretion when studying women of premenopausal age.

GERTRUDE D. MAENGWYN-DAVIES, PH.D.†
RUTH WEINER, B.S.

Wilmer Institute,
The Johns Hopkins Hospital and University,
Baltimore, Maryland

REFERENCES

1. PORTER, C. C., and SILBER, R. H.: A quantitative color reaction for cortisone and related 17,21-dihydroxy-20-ketosteroids, *J. Biol. Chem.* **185**: 201. 1950.
2. GLENN, E. M., and NELSON, D. H.: Chemical method for the determination of 17-hydroxycorticosteroids and 17-ketosteroids in urine following hydrolysis with β-glucuronidase, *J. Clin. Endocrinol. & Metab.* **13**: 911, 1953.

† At present: Associate Research Professor, Dept. of Pharmacology, The George Washington University Medical School, Washington 5, D. C.

3. SANDBERG, A. A.; NELSON, D. H.; GLENN, E. M.; TYLER, F. H., and SAMUELS, L. T.: 17-Hydroxycorticosteroids and 17-ketosteroids in the urine of human subjects; clinical application of a method employing β-glucuronidase hydrolysis, *J. Clin. Endocrinol. & Metab.* **13**: 1445, 1953.
4. NELSON, D. H.: Personal communication.
5. EDWARDS, A. L.: Statistical Analysis, ed. 4. New York, Rinehart and Co., Inc., 1950, p. 330.

ATYPICAL PORTER-SILBER REACTION AFTER THE ADMINISTRATION OF SULFAMERAZINE

TO THE EDITOR:

When the technique of Reddy, Jenkins and Thorn (1, 2) for the determination of urinary 17-hydroxycorticoids is applied to the crude butanol extracts of urine of patients who have been treated with sulfamerazine, an orange to orange-brown color of the final solution is uniformly observed.

Figure 1 shows the absorption spectrum of the colored reaction products resulting from phenylhydrazine/sulfuric-acid treatment of the butanol extract of the urine of an 18-year-old girl, suffering from asthma bronchiale, who was treated with 4 Gm. of sulfamerazine daily during an attack of pneumonia.

Figure 1 also shows the absorption spectra of the reaction products in the extracts of urine of a normal male, both before and after administration of 4 Gm. of sulfamerazine in one day. The measurements were made on a Coleman Jr. spectrophotometer, model 6A. They were corrected for the color produced when the urinary butanol extracts are treated with sulfuric acid alone, as recommended by Porter and Silber (3).

Crystalline sulfamerazine does not cause a color reaction with the phenylhydrazine/sulfuric-acid reagent, neither does acetyl-sulfamerazine. (We tried this latter substance, since it is known, that sulfamerazine is excreted partly as the acetyl-derivative.)

The same normal male who took 4 Gm. of sulfamerazine agreed to take 2 and 4 Gm. daily of sulfanilamide; no atypical color developed when we applied the technique of Reddy *et al.* to the butanol extracts of the urine voided during this period. The latter finding seems to indicate, that the sulfanilamide grouping in the sulfamerazine molecule does not account for the development of the orange color.

The absorption band of the atypical chromogen covers the absorption region of the typical Porter-Silber chromogens. When therapeutic doses of sulfamerazine are administered, the absorption caused by the atypical chromogen is so predominant (Fig. 1) that it is impossible to eliminate it

by using a correction formula based on spectrophotometer readings at different wavelengths.

We therefore agree with the opinion of Marks and Leftin (4), that the clinical application of the method of Reddy, Jenkins and Thorn for the

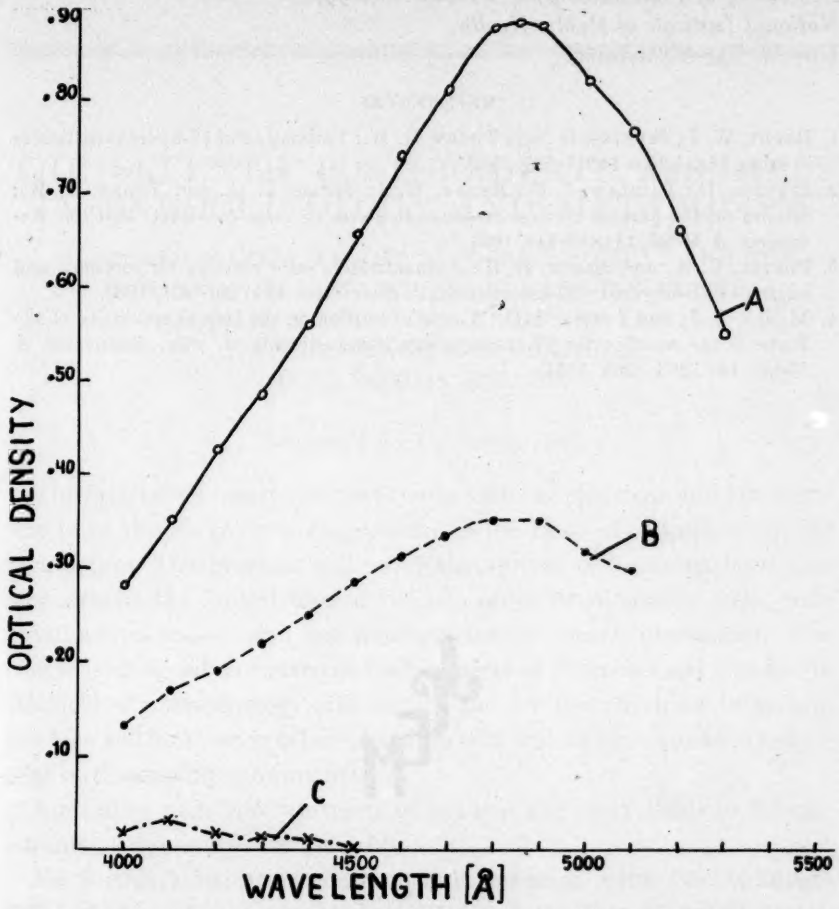

FIG. 1. Absorption spectrum of colored reaction products observed following addition of phenylhydrazine/sulfuric acid to 1 ml. butanol extract (≈ 1 ml. urine) of:

A. A patient treated with 4 Gm. of sulfamerazine daily during an attack of pneumonia.

B. A normal male who took 4 Gm. of sulfamerazine on the day preceding the collection of urine.

C. The same subject during a period when no sulfamerazine was administered.

determination of corticoids in urine is valid only under carefully controlled conditions.

D. A. V. M. LAMPE-HINTZEN, M.D.
L. G. HUIS IN 'T VELD, PH.D.
Laboratory of Pharmacology and Endocrinology,
National Institute of Public Health,
Utrecht, The Netherlands

REFERENCES

1. REDDY, W. J.; JENKINS, D., and THORN, G. W.: Estimation of 17-hydroxycorticoids in urine, *Metabolism* **1**: 511–527, 1952.
2. JENKINS, D.; LAIDLAW, J. C.; REDDY, W. J.; MILES, L. C., and THORN, G. W.: Studies on the adrenal cortical response to stress in man, (abstract) *J. Clin. Endocrinol. & Metab.* **13**: 843–844, 1953.
3. PORTER, C. G., and SILBER, R. H.: A quantitative color reaction for cortisone and related 17,21-dihydroxy-20-ketosteroids, *J. Biol. Chem.* **184**: 201–207, 1950.
4. MARKS, L. J., and LEFTIN, J. H.: A note of caution on the lack of specificity of the Porter-Silber reaction for 17,21-dihydroxy-20-ketosteroids, *J. Clin. Endocrinol. & Metab.* **14**: 1263–1265, 1954.

The Endocrine Society

THE SEVENTH POSTGRADUATE ASSEMBLY IN ENDOCRINOLOGY AND METABOLISM

SPONSORED JOINTLY BY THE ENDOCRINE SOCIETY AND THE INDIANA UNIVERSITY SCHOOL OF MEDICINE AT THE CONTINUATION STUDY CENTER OF THE INDIANA UNIVERSITY MEDICAL CENTER, INDIANAPOLIS, INDIANA

September 26–October 1, 1955

The faculty will consist of twenty-one eminent clinicians and investigators from various parts of the country in the fields of endocrinology and metabolism. The program will cover the various endocrinopathies, with emphasis on the clinical aspects, demonstration of laboratory tests, presentations of cases, and question-and-answer panel discussions. The course is designed to cover the main aspects of diagnosis and therapy in the field of endocrinology and metabolism for the physician in general practice and for those in other specialties who wish to have a general knowledge of this rapidly growing field.

A syllabus with brief abstracts of lectures will be available to the registrants at the time of the assembly.

For further information concerning the program, write Post Graduate Office, Indiana University Medical Center, 1100 West Michigan Street, Indianapolis 7, Indiana. Registration is limited to 100; tuition fee is $100.00. Arrangements for housing accommodations will be made through the Indiana Medical Center.

(For program, see July issue)

THE 1956 AWARDS

The selection of the recipients of the awards of The Endocrine Society is made by a Committee appointed by the Council of the Society. These awards and fellowships carry no obligation by the recipient to the Society or to the donors.

MEDAL OF THE ENDOCRINE SOCIETY

In 1954 the Council of the Society voted to establish a medal and an honorarium of $1,000 to be given to an individual for work of special distinction in endocrinology. The recipient shall be chosen from nominations presented by members of the Society and is limited to citizens of the United States and Canada.

THE CIBA AWARD

The Ciba Award, to recognize the meritorious accomplishments of an investigator not more than 35 years of age in the field of clinical or preclinical endocrinology, was established in 1942, but no recipient was selected in 1942 or 1943. In 1944 the Award was presented to Dr. E. B. Astwood; 1945—Dr. Jane A. Russell; 1946—Dr. Martin M. Hoffman; 1947—Dr. Choh Hao Li; 1948—Dr. Carl Heller; 1949—Dr. George Sayers; 1950—Dr. Oscar M. Hechter; 1951—Dr. Albert Segaloff; 1952—Dr. Seymour Lieberman; 1953—Dr. Sidney Roberts and Dr. Clara Szego (Mrs. Roberts); 1954—Dr. Isadore M. Rosenberg; 1955—Dr. Jack Gross. Prior to 1952 the Award was $1,200. It is now $1,800. If within twenty-four months of the date of the award, the recipient should choose to use it toward further study in a laboratory other than that in which he is at present working, it will be increased to $2,500.

THE AYERST, McKENNA AND HARRISON FELLOWSHIP

This Fellowship was established in 1947. It is designed to assist men or women of exceptional promise in furthering their advancement towards a career in endocrinology. The Fellowship is awarded on alternate years (1955, 1957, etc.) and the stipend, which will not exceed $5,000, may be divided into two Fellowships in varying amounts in accordance with the qualifications of the appointees. Individuals possessing the M.D. or Ph.D. degree, or candidates for either of these degrees, are eligible for appointment.

Applicants must submit the following information:

1. Evidence of scientific ability as attested by studies completed or in progress.

2. Recommendations from individuals familiar with the candidate and his work.
3. A proposed program of study.
4. Acceptance of the individual by the head of the department in which the Fellowship will be held.
5. A statement that he or she will serve full time if awarded a Fellowship. A small amount of time (10 to 15 per cent) may be spent in course work or participation in teaching, the latter purely on a voluntary basis.

THE SCHERING AND THE UPJOHN SCHOLARS OF THE ENDOCRINE SOCIETY

The Council of The Endocrine Society has established a category of Scholars. These grants have been made available through the generosity of the Schering Corporation and the Upjohn Company, and will be awarded to established investigators and teachers in the field of endocrinology who wish to extend their opportunities for work either in this country or abroad.

The awards will not exceed $2,500 annually for each individual and will be granted on the basis of proposals submitted by the applicant. Such applications should include the estimated financial needs. The funds may be used for travel, maintenance and other expenses.

Nominations

Nominations for the Medal of the Endocrine Society; the Ciba Award; and the Ayerst, McKenna and Harrison Fellowship may be made by any member of the Endocrine Society. They should be submitted on forms which may be obtained from the Office of the Secretary, 1200 North Walker Street, Oklahoma City 3, Oklahoma. Completed nominations should be returned to the Secretary not later than *November 1* each year.

Proposals for appointments as Scholars of The Endocrine Society should be made in writing by the individual, and addressed to the Secretary of the Society. They should be submitted by *November 1* each year.

The Awards Committee will meet in November and notice of awards to successful nominees and applicants will be made not later than December 1.

BOOK REGISTER

ABC für Zuckerkranke. Ein Ratgeber für den Kranken, ed. 7. By PROF. DR. F. BERTRAM, Chefartz der II. medizinischen Klinik des Allgemeinen Krankhauses Barmbek, Hamburg, Germany; 76 pages, 5 figures, 9 tables, 1955. Stuttgart, Georg Thieme Verlag. D.M. 4.20. In U.S.A. and Canada, Intercontinental Medical Book Corp., New York 16. Price $1.00.

American Lectures in Endocrinology. Edited by Willard O. Thompson, M.D. Published by Charles C Thomas, Publisher, Springfield, Illinois.

El diagnostico y tratamiento de la insuficiencia suprarrenal. (The Diagnosis and Treatment of Adrenal Insufficiency), segunda edicion. By GEORGE W. THORN, M.D., Hersey Professor of the Theory and Practice of Medicine, Harvard Medical School; Physician-in-Chief, Peter Bent Brigham Hospital, Boston, Massachusetts; 214 pages; 35 figures; copyright 1955. CARLOS TANTURI, M.D., Editor, Buenos Aires. El Ateneo, Buenos Aires.

Sexual Precocity. By HUGH JOLLY, M.A., M.D.(Camb.), M.R.C.P., Consultant Paediatrician. Plymouth Clinical Area; Late Senior Medical Registrar, Hospital for Sick Children, London, England. Foreword by LAWSON WILKINS, M.D., Assoc. Professor of Pediatrics, The Johns Hopkins University, Baltimore, Md.; 304 pages; 91 illustrations; 1955. Price $6.75.

Annotated Bibliography, Vitamin E. Volume III (1952–1954). Compiled by PHILIP L. HARRIS and WILMA KUJAWSKI; 182 pages; 1955. Distributed by The National Vitamin Foundation, Inc., 15 East 58th Street, New York 22, N. Y.

Antimetabolites and Cancer. Edited by CORNELIUS P. RHOADS, M.D. A Symposium presented on December 28–29, 1953 at the Boston meeting of the A.A.A.S.; 318 pages; 50 illustrations; 1955. Washington, D. C., The American Association for the Advancement of Science. Price:—regular $5.75—prepaid to A.A.A.S. members $5.00.

Ciba Foundation Colloquia on Ageing. Volume I. Ageing—General Aspects. Edited by G. E. W. WOLSTENHOLME, O.B.E., and MARGARET P. CAMERON, M.A.; 268 pages; 38 illustrations; 1955. There are chapters dealing with the mental aspects of ageing, 17-KS excretion in ageing subjects, too rapid maturation in children as a cause of ageing, and adrenocortical reactivity in aged schizophrenic patients. Boston, Mass.; Little, Brown and Company. Price $6.75.

Ciba Foundation Colloquia on Endocrinology. Volume VIII. The Human Adrenal Cortex. Edited by G. E. W. WOLSTENHOLME, O.B.E., and MARGARET P. CAMERON, M.A. Part I deals with histologic and biochemical aspects and cortico-medullary relationships—Chairman, DR. GREGORY PINCUS, Director of Laboratories, Worcester Foundation for Experimental Biology, Shrewsbury, Mass. Part II deals with physiologic and pathologic aspects and hypothalamic and pituitary relationships—Chairman, DR. GEORGE W. THORN, Physician-in-Chief, Peter Bent Brigham Hospital, Boston, Mass.; 653 pages; numerous illustrations; 1955. Price $10.00.

Clinical Endocrinology, ed. 2. by LAURENCE MARTIN, M.D., F.R.C.P., Physician to Addenbrooke's Hospital, Cambridge, England, and MARTIN HYNES, M.D., M.R.C.P., Clinical Pathologist to the Royal Northern Group of Hospitals, with a Foreword by SIR LIONEL WHITBY, M.D., F.R.C.P., Regius Professor of

Physic, University of Cambridge; 261 pages; 39 figures; 1955. Boston, Mass.; Little, Brown and Company. Price $5.50.

Endocrinologia, ed. 3. por el PROF. RODOLFO Q. PASQUALINI. Professor Titular de Clinica Médica y del Curso de Endocrinologia para Graduados de la Facultad de Medicina de Buenos Aires; Director del Instituto de Endocrinologia; 620 pages; profusely illustrated; 1955. Buenos Aires, El Ateneo.

Endocrinologia sessuale dal sesso all'intersesso. TEUCLO DATI. Prefazione di GIOVANNI DI GUGLIELMO; 986 pages; 15 chapters; 174 illustrations; 1955. Rome, Italy, Il Pensiero Scientifico Editore. Lire 6000.

Endocrinologie sexuelle du foetus féminin. P. ROSA, Adjoint au Service de Gynécologie et d'Obstétrique de l'Université libre de Bruxelles. Préface du PROF. J. SNOECK; 316 pages; 100 figures; 1955. Paris, France, Masson et Cie, Éditeurs. Price—2800 fr.

L'année endocrinologique. Septième annèe. Par M. ALBEAUX-FERNET, L. BELLOT, P. BUGARD, J. CHABOT, J. DERIBREUX, A. FORBES, M. GELINET, J. GUENEL, C.-M. LAUR et J.-D. ROMANI; 248 pages; illustrated; 1955. Paris, France, Mason et Cie, Éditeurs.

Comment traiter les aménorrhées. Par ALBERT NETTER, Médecin des Hôpitaux de Paris; 100 pages; 1955. Paris, France, Éditions Médicales Flammarion.

Reserpine in the Treatment of Neuropsychiatric, Neurological, and Related Clinical Problems. Papers presented at a conference held by the Section of Biology of the New York Academy of Sciences, February 3 and 4, 1955. Annals of the New York Academy of Sciences, Volume 61, pp. 1–280, April 15, 1955, published by the Academy. Price $3.50.

Sexual Hygiene and Pathology. A Manual for the Physician. By JOHN F. OLIVEN, M.D., Psychiatrist to Vanderbilt Clinic, Columbia-Presbyterian Medical Center, New York. A professional book for distribution only to professional people; 494 pages; 1955. Philadelphia, Pa., J. B. Lippincott Company. Price $10.00.

Studies on Expenditure of Energy and Consumption of Food by Miners and Clerks, Fife, Scotland, 1952. By R. C. GARRY, R. PASSMORE, GRACE M. WARNOCK and J. V. G. A. DURNIN. Medical Research Council Special Report Series No. 289; 76 pages; 10 figures; 37 tables; 1955. London, Her Majesty's Stationery Office. Price, 5 shillings net.

Textbook of Medicine, edition 9. Edited by RUSSELL L. CECIL, M.D., Professor of Clinical Medicine Emeritus, Cornell University, New York, and ROBERT F. LOEB, M.D., Bard Professor of Medicine, Columbia University, New York. This edition contains many articles on subjects not covered in previous editions; other articles have been brought up to date; 103 pages are devoted to diseases of the ductless glands; 1810 pages; over 200 illustrations; 1955. Philadelphia, Pa., W. B. Saunders Company.

The Technique and Significance of Oestrogen Determinations. Memoirs of the Society for Endocrinology, No. 3. Edited for the Society by P. ECKSTEIN and S. ZUCKERMAN; 96 pages; illustrated; 1955. New York, Cambridge University Press. Price $3.75.

Transactions of the American Goiter Association for 1954. A collection of papers dealing with the latest clinical and physiologic advances in the study of the thyroid gland, by outstanding authors in the field; 496 pages; 204 illustrations; 1955. Springfield, Illinois, Charles C Thomas, Publisher. Price $14.00.

Transactions of the Josiah Macy, Jr. Foundation Conferences. Publications Sales Office, P.O. Box, Packanack Lake, New Jersey.

Neuropharmacology. First Conference, May 26–28, 1954, Princeton, N. J. Edited by HAROLD A. ABRAMSON, M.D., Assistant Clinical Professor of Physiology, Columbia University College of Physicians and Surgeons, New York, N. Y.; 209 pages; 44 figures; 15 tables; 1955. Price $4.25.

Problems of Consciousness. Fifth Conference, March 22–24, 1954, Princeton, N. J. Edited by HAROLD A. ABRAMSON, M.D., Assistant Professor of Physiology, Columbia University College of Physicians and Surgeons, New York, N. Y.; 180 pages; 10 figures; 1955. Price $3.50.

Tumors of the Thymus Gland. (Section V—Fascicle 19: Atlas of Tumor Pathology.) By BENJAMIN CASTLEMAN, M.D., Chief, Department of Pathology and Bacteriology, Massachusetts General Hospital, and Clinical Professor of Pathology, Harvard Medical School, Boston, Massachusetts; 81 figures and 4 color plates; 1955. Published by the Armed Forces Institute of Pathology under the auspices of the Subcommittee on Oncology of the Committee on Pathology of the National Research Council, For sale by the American Registry of Pathology, Armed Forces Institute of Pathology, Washington 25, D. C. Price $1.00.